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The development of fermented pea protein-coconut milk beverage

A thesis submitted in partial fulfilment of the requirement for the degree of Master of Food Technology

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ABSTRACT

Most commercially available probiotic products are dairy-based and are associated with consumer health challenges such as lactose intolerance and allergenicity due to milk proteins. Therefore, a strong consumer interest in searching for alternative products and ingredients that can deliver similar health benefits to dairy-based products. Plant protein is an important nutrient for the normal growth and functioning of the human body. Pea protein is of interest to food manufacturers due to its high nutritive value. However, it is characterised by a strong off-flavour (beany flavour) making it difficult to formulate into acceptable consumer food products. The main aim of this study was to reduce the beany off-flavour of commercial pea protein powder intended for the production of an organic fermented pea protein-coconut milk beverage. This research was conducted in three main phases.

In Phase 1, salt extraction and isoelectric precipitation methods were used to further purify commercial pea protein powder (Roquette S85F, France). After purification, the yellow commercial pea protein powder ($b^* = 16.32 \pm 0.09$) had transformed into a white pea protein paste ($b^* = 6.86 \pm 0.12$).

Phase 2 investigated the reduction of the beany off-flavour by fermentation of a novel fermented pea protein-coconut milk beverage. In this phase, the refined pea protein paste (phase 1) was added to organic coconut milk (Ceres Organics, Auckland) and then fermented by a mixed lactic culture (VEGE 053 LYO). The single factor test and orthogonal experimental design were used to determine the optimum fermentation conditions of the fermented pea protein-coconut milk beverage. In these experiments, three fermentation temperatures (37 °C, 40 °C, 43 °C) with three protein concentrations (3%, 5%, 7%, w/v) and three fermentation times (8, 10 and 12 h) were used to conduct nine experimental treatments (formulations). The three best fermented beverages were selected based on viable cell counts (VCCs) and sensory evaluation by a semi-trained sensory panel (n=18). These three best samples were further evaluated by a consumer sensory panel (n=90). The fermented beverage containing 3% pea protein and fermented at 40 °C /8 h was evaluated as the best product by the consumer sensory panelists. The final selected formulation had the highest viable cell counts (8.78±0.21 log CFU /mL) and overall mean sensory acceptability scores (6.2±0.50). Other parameters determined in the final formulation of the fermented beverage were pH, titratable acidity (T.A.), colour and crude protein. During fermentation for 8 h, the pH decreased from 6.15±0.13 to 4.29±0.02, while the T.A. increased from $0.09\% \pm 0.01$ to $0.52\% \pm 0.03$. Colour changed significantly (p<0.01), whereas there was no significant (p>0.05) difference in the protein content of the fermented pea protein-coconut milk beverage during fermentation.

In the third phase, the stability of the fermented beverage during storage (4 °C) for 21 days, was determined by measuring pH and colour as well as the analysis of protein and sensory characteristics. A semi-trained sensory panel (n=15) evaluated the fresh and stored beverage for various sensory characteristics including overall acceptance using the 9-point hedonic scale. During storage of the beverage, the pH, titratable acidity, cell counts and colour changed significantly (p<0.05). By the end of storage, the pH had decreased from 4.43 ± 0.03 to 4.38 ± 0.02 (p<0.05), while T.A. increased slightly. The sensory characteristics were stable during storage. Despite the changes in the physical-chemical characteristics of the fermented beverage, the product was still found to be acceptable by a semi-trained sensory panel following storage for 21 days.

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LIST OF ABBREVIATIONS

a*	=	redness-greenness
ANOVA	=	Analysis of variance
AOAC	=	Association of Official Analytical Chemist
ATP	=	Adenosine triphosphate
b*	=	yellowness-blueness
B.C.	=	before Christ
BCAAs	=	branch chain amino acids
BCE	=	Before the Common Era
Ca	=	Calcium
CaCl ₂	=	Calcium chloride
CaCO ₃	=	Calcium carbonate
CFU /mL	=	colony-forming units per milliliter
CPPP	=	Commercial pea protein powder
DCU	=	Direct Culture Unit
etc	=	et cetera
F6PPK	=	fructose-6-phosphate phosphoketolase
g	=	gram
Ğ	=	Gram
GC-MS	=	gas chromatography-mass spectrometry
GLM	=	general linear model
GMO	=	genetically modified organism
h	=	Hours
HC1	=	hydrochloric acid
HPLC	=	high performance liquid chromatography system
HDL	=	high-density lipoprotein cholesterol
L	=	Litre
 L*	=	Lightness
LAB	=	Lactic acid bacteria
LDL	=	low-density lipoprotein cholesterol
LOX	=	Lipoxygenase
LSD	=	Least significant difference
K_2SO_4	=	Potassium sulphate
Min	=	Minute
mg	=	milligram
mL	=	millilitre
MCTs	=	medium-chain triglycerides
MRS	=	De Man Rogosa and Sharpe agar
NaOH	=	sodium hydroxide
P	=	phosphorus
рН	=	potential hydrogen
Se	_	Selenium
SD	=	standard deviation
ТА	=	titratable acidity
UHT	=	Illtra-high temperature
UV	_	Illtra Violet
VCCs	=	Viable cell counts
WHO	=	World Health Organization
w /v	=	weight per volume
11 o	_	microgram
r6	_	morogram

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1. INTRODUCTION

1.1 Background

Consumer awareness of health and nutrition has markedly increased in the last two decades (Abdel- Rahman, Eltayeb, Azza, & Feria, 2011). Of the ingredients in functional foods, protein is an essential nutrient for health growth and functioning of the human body. Despite the importance of this macronutrient, world protein requirements continue to be a global issue with heightened concerns about food security and protein malnutrition. In 1997 the Food and Agriculture Organisation of the United Nations (FAO) estimated that over 800 million people in the developing world were undernourished (Blandford & Viatte, 1997), and the number continues to increase. As an essential nutrient element in the body, proteins are made of 20 amino acids, some of which can be synthesised in the human body while others must be obtained from food (essential amino acids). Protein is found in both animal and plant sources. Animal sources food which contain high protein content includes meat, seafood, eggs and milk products. Plant protein is mainly found in legumes (peas, beans and lentils), grains (quinoa, oats and barley) and nuts. However, there is increasing evidence that a diet mainly based on animal protein may be linked to indigestion, high blood pressure and heart disease, because of the consumption of high saturated fat (Kaluza, Åkesson, & Wolk, 2015; Choi et al., 2013). Plant proteins are good sources of dietary fiber and are generally low in fat, particularly saturated fats. Diets high in plant protein, such as the vegetarian diet, are associated with many health benefits such as low body weight, low cholesterol and balance blood pressure (Craig, 2010). Therefore, high nutrition value plant-based protein represents a suitable dietary substitution to animal-based protein (Duranti & Gius, 1997). As the demand for proteins in the world is increasing, the need for high-quality protein derived from plants is particularly strong.

Legumes (peas, chickpeas, lentils and beans) are considered inexpensive sources of proteins for low-income groups of the population and are commonly used substitutes for meat. If their application in the food industry can be expanded, legumes can play a significant role in alleviating protein-energy malnutrition (Mateos-Aparico, Redondo, Villanueva-Suarez, & Zapata-Revilla, 2008). Legumes constitute an important source of dietary protein for large segments of the world's population, especially in those countries in which the consumption of animal proteins is limited due to either its non-availability or higher price (Boye, Zare, & Pletch, 2010).

The pea (*Pisum sativum*) is an important legume which has been grown since the beginning of arable farming (7000–6000 B.C.). In New Zealand, it is considered the third most important cash crop of all the legumes (Jermyn, 1983). Growing peas can improve soil fertility as they fix nitrogen into the soil through their roots, reducing the need for chemical fertilizers. Peas are commonly consumed as seeds, flour and protein isolate (Aiking, de Boer, & Vereijken, 2006) which are relatively low in calories and contain several vitamins, minerals and antioxidants. Pea products are also high in fiber and protein (Muehlbauer & Tullu, 1997), making them important to the human diet (White, 1983). However, the commercial application of the pea (especially the yellow pea) is limited by its undesirable colour, unpleasant taste and flavour and low solubility. Therefore, based on available reports (Hansen, Jakobsen, & Christensen, 2000; Murat, Bard, Dhalleine, & Cat, 2013), it is challenging to overcome the undesirable characteristics.

The dark yellow colour of pea protein can be improved by using different refining methods which involve isoelectric precipitation and salt extraction. After extraction, the colour of commercial pea protein powder changes from dark yellow to white and the particle size will be smaller (Scopes, 2013).

The bitter, beany taste and the "green", "grassy", "hay-like" odor profile of pea protein are the second significant barriers to its application. There are many causes of unpleasant taste and flavour, which include lipid oxidation and lipoxygenase activities. The main volatile compounds in pea protein products connected with the off-flavour are pentanol, hexanol, heptanol, hexanal, and ethyl vinyl ketone (Wang et al., 1997). Several methods have attempted to reduce the most potent odor-active volatiles in pea protein preparations. Such as temperature controlling, cyclodextrins entrapping and flavour masking (Hashimoto, 2004; Suratman et al., 2004; Lan et al., 2019). Except for these physical and chemical methods, the reduction of beany flavour can also be achieved by lactic acid bacteria (LAB) fermentation.

LAB fermentation has been used since ancient times to preserve perishable food materials (McGovern, 2013). Fermentation is the process of converting carbohydrates to alcohol or organic acids using microorganisms. Widely consumed fermented foods include yoghurt, vinegar, olives, and cheese. More foods prepared by fermentation may also be based on beans,

grain, vegetables, fruit, honey, dairy products, fish, meat, or tea (Gupta & Abu-Ghannam, 2012). With respect to reduction of the beany flavour by fermentation, many researchers agree that technology could remove or reduce the off-flavour of legume protein (Moy, Lu, & Chou, 2012; Schindler et al., 2012), which is commonly attributed to change or decrease of volatile compounds in extracted protein (Kaneko, Kumazawa, & Nishimura, 2011; Shi et al., 2015). During the fermentation, complex organic compounds are broken down into smaller molecules by lactic acid microorganisms (Schindler et al., 2012). Lactic acid bacteria that ferment food comprise of a large number of species as such as *Streptococcus thermophilus, Lactobacillus delbrueckii* subsp. bulgaricus, *Lactobacillus acidophilus*, and *Lactobacillus helveticus*. Probitioc bacteria such the *Bifidobacterium* sp. also participate in lactic fermentation, although their growth is slow. Therefore, lactic acid fermentation has been widely used in various pea seeds and pea protein modification to improve the sensory and functional properties of the final products (Marilley et al., 2004; Moslehishad et al., 2013; Czarnecka et al., 1998).

The low solubility, especially insolubility at low pH is another problem associated with the application of pea protein in fermented food. Coconut milk has a rich and creamy texture (Belewu & Belewu, 2007), and when pea protein and coconut milk are mixed, the pea protein particles can be wrapped in the fat granules of the coconut milk and thus become suspended in the solution. The fermentation of pea protein and coconut milk mixture will increase the solubility of the pea protein at low pH and produce a more uniform texture for the fermented pea protein-coconut milk beverage. Furthermore, coconut milk has a very aromatic smell which could help to mask and reduce the beany flavour of the pea protein.

1.2 Aim and Objectives

Aim:

The main aim of this project was to reduce the yellowness and reduce the beany flavour of the commercial pea protein powder for the production of a new fermented pea protein-coconut milk beverage.

Objectives:

- 1. To improve the colour and beany flavour of commercial pea protein powder using isoelectric precipitation and salt extraction methods;
- 2. To use orthogonal data analysis to optimise fermentation conditions of pea protein-coconut milk fermentation;
- 3. To produce a stable prototype fermented pea protein-coconut milk beverage through evaluating the physicochemical and sensory properties.

2. LITERATURE REVIEW

2.1 Introduction

Over the last two decades, there has been an increasing demand for healthy and protein-rich foods (Chacón-Lee & González-Mariño, 2010; Abdel-Rahman, Eltayeb, Azza, & Feria, 2011), as protein is an important nutrient required for the healthy growth and functioning of the human body. There have two kinds of protein sources, animal and plant. Animal protein sources are considered to be complete sources of protein because they contain all of the essential amino acids that your body needs to function effectively. In contrast, plant protein sources are considered to be incomplete, as they lack one or more of the essential amino acids that your body needs (Hoffman & Falvo, 2004). Therefore, to achieve a balanced amino acid intake, consumption of a variety of plant protein sources is required, so that the amino acid compositions complement each other in the diet. However, there is increasing evidence that a diet primarily based on animal protein is associated with various diseases in humans such as high blood pressure and heart diseases (Kaluza, Åkesson, & Wolk, 2015; Choi et al., 2013), mostly due to the high-fat content of animal protein. On the other hand, plant protein sources are generally good sources of dietary fibre and low in fat content, particularly saturated fats. Diets high in plant proteins, such as the vegetarian diet, are linked with many health benefits, with studies suggesting that vegetarians tend to have lower body weight, lower cholesterol and lower blood pressure levels than meat lovers. They also have a lower risk of stroke, cancer and death from heart disease than non-vegetarians (Craig, 2010). Therefore, plant-based represents a respectable dietary substitution to animal-based protein and it has much more health benefits for people than animal protein (Duranti & Gius, 1997; Kate, 2011).

Legumes (soybeans, peas and lentils) are widely known as important sources of food and feed proteins around the world (Weisse et al., 2009). Because of the high protein quantity of legume, and the benefits of consumption legume protein isolate have a great number of references (Duranti, 2006; Nunes, Raymundo, & Sousa, 2006; Duranti & Gius, 1997; Anderson et al., 1999; Rebello, Greenway, & Finley, 2014). It is recognized that the legume protein isolate offers the immense possibility in the development of a new class of plant protein food.

2.2 The pea and pea protein

2.2.1 Pea

Pea (*Pisum sativum*) is an herbaceous vine that belongs to the family of Fabaceae with common names, including English peas, sweet peas, and garden peas (Uebersax & Ruengsakulrash, 1989). *Pisum* is one of the oldest domesticated plants in the world. Carbonized pea seeds found in Neolithic settlements in Iraq, Turkey, and Greece (7000–6000 B.C.) have been attributed to domesticated peas, which indicates that peas have been cultivated for as long as barley and wheat (Zohary & Hopf, 1973). Besides soy, peanut, and bean, field peas constitute a significant sector of agricultural grain production, with approximately 25 million hectares grown annually worldwide (Vankosky et al., 2011).

Peas thrive in well-drained, sandy soil supplemented with adequate moisture. The seed can be planted at 10 °C, however, it grows faster at temperatures of 13 to 18 °C. Many cultivars reach maturity around 60 days after planting (Maiti et al., 2012) with each pod measuring about 5-8 cm in length and filled with smooth edible seeds (Figure 2.1). Peas are also grown to improve soil fertility as they can fix nitrogen into the soil through their roots, reducing the requirement for chemical fertilisers (Chapagain & Riseman, 2014).



Figure 2.1 The plant (a) and seeds (b) of pea (*Pisum sativum*)

Note: Source of the figures-Google image

2.2.1.1 Pea species

There are six species of pea seeds. Figure 2.2 describes the characteristics of pea plants. Peas have been grown in New Zealand since the beginning of arable farming, and are the third most important annual cash crop in New Zealand. Canterbury is the most popular area for pea growing, with Hawkes Bay also being a major producer of vining peas (White, 1983).

	Rices	1.40°	Grenkter		Varyabar		Arger		Karcoviz		interest
Common name	Process peas, vegetable peas, edible podded peas	Common name	Green/blue field peas	Common name	White/yellow field peas	Common name	Maple field peas	Common name	Marrowfat field peas	Common name	Forage peas
Grain characteristics	Wrinkled green cotyledoned grain with a clear - green seed coat.	Grain characteristics	Smooth/spherical blue- green cotyledoned grain with a transparent seed coat.	Grain characteristics	Smooth – slightly wrinkled yellow cotyledoned grain with a transparent seed coat.	Grain characteristics	Irregular/dimpled brown mottled grain with a yellow cotyledon.	Grain characteristics	Large, irregular, grain with green cotyledon and clear seed coat.	Grain characteristics	Medium sized tan/ brown or green coloured grain with a yellow or green cotyledon.
Maturity range	Early (9 nodes to first flower) to 17 nodes.	Maturity range	Usually mid-season, flowering around 15-17 nodes,	Maturity range	Mid-season maturity with node to first flower from 15-17 nodes.	Maturity range	Mid-late maturity with first flowering node usually from 16-18 nodes.	Maturity range	Mid season maturity with first flower around 15-16 nodes.	Maturity range	Usually late and indeterminate maturity to synchronise with cereal maturity.
Leaf characteristic	Mix of leafed and semi-leafless varieties available.	Leaf characteristic	Almost exclusively semi- leafless plant types.	Leaf characteristic	Apart from Kornet, all are semi-leafless.	Leaf characteristic	Both tall indeterminate varieties (eg Whero) and shorter, more determinate forms (eg Courier).	Leaf characteristic	Generally marrowfat varieties are short and leafed although new semi-leafless varieties are becoming popular.	Leaf characteristic	Both leafed and semi-leafless varieties available. Varieties are tall and highly productive in relation to dry matter with a low grain harvest index.
Typical sowing window	Large range from late July through to early December.	Typical sowing window	September to end of October.	Typical sowing window	September to end of October.	Typical sowing window	August to end of October.	Typical sowing window	September to end of October.	Typical sowing window	May to end of October.
Typical end use	Freezing, dehydration, canning, fresh vegetable sold as unthreshed pods, low fibre and large podded varieties (eg snow peas) consumed frozen and fresh.	Typical end use	Split peas for soup, canning, whole seed and extruded snack production, rolling.	Typical end use	Food use for split peas for soup, canning, extruded snack products, pea flour, animal feed.	Typical end use	Predominantly used for bird feed industry. Undesirable for animal feed due to high tannin and anti-nuthtonal compounds such as trypsin inhibitor. Used also for food sprouts.	Typical end use	Food use in high value snack production in Japan and S.E. Asia. Premiums are paid for high quality colour, and uniform large grain.	Typical end use	Forage use: can be grown as a stand alone forage or slage crop or in combinations with cereals.
Domestic and/ or export	Mix of export and domestic use.	Domestic and/ or export	Majority exported for food use.	Domestic and/ or export	Majority are exported.	Domestic and/ or export	Almost exclusively exported.	Domestic and/ or export	Almost exclusively exported.	Domestic and/ or export	Domestic use.
Organic production possible?	Yes.	Organic production possible?	Yes.	Organic production possible?	Yes,	Organic production possible?	Yes.	Organic production possible?	Yes.	Organic production possible?	Yes.
Major varieties	Ashton, Bolero, CFR Sonata, CFR Pinacle	Major varieties	Aragorn, Ariel, Crusader, Prussian Blue.	Major varieties	Alezan, Komet, Miami, Santana.	Major varieties	Courier, Whero.	Major varieties	Midichi, Midlea, Primo.	Major varieties	AP2, Magnus, Provider.

Figure 2.2 Different types of peas and their characteristics (Greenwood, Aves, & Catherwood, 2008).

2.2.1.2 Nutritional value of the pea

Peas are an excellent source of a variety of health-promoting nutrients, such as vitamins, minerals, carbohydrates, proteins and soluble and insoluble fibre (Rubio et al., 2014).

2.2.1.2.1 Vitamins

Peas are important sources of vitamins, including Vitamin-C, Vitamin-A, Vitamin-K and Vitamin-B (Duranti & Gius, 1997) (Table 2.1). Consumption of natural vegetables /fruits rich in these vitamins has many benefits for the human body. Vitamin-C can scavenge harmful, pro-inflammatory free radicals from the body and help improve resistance against infectious agents (Jacob & Sotoudeh, 2002). Vitamin-A helps to protect from oral cavity and lung cancers (Stephensen, 2001). Vitamin-K can restrict neuronal damage in the brain, and hence it has an established role in the relief of Alzheimer's disease (Olson, 1984). Vitamin B6 consumption before pregnancy helps prevent neural tube defects in the foetus (Ogawa et al., 1991).

Vitamin	Concentration /100 g pea protein	Proportion of daily recommended intake (%)	Reference
Vitamin-C	40 mg	60	Alonso et al., 2000
Vitamin-A	25.5 mg	4	Alonso et al., 2000
Vitamin-K	24.8 µg	3	Makhlouf et al., 1995
Vitamin-B6	65 µg	5	Igbasan & Guenter, 1996

Table 2.1 Vitamin content of pea protein

2.2.1.2.2 Minerals

Peas contain several important minerals, including calcium, iron, zinc, magnesium, copper, phosphorus, manganese and potassium. Of these, the most abundant is manganese. 100-g pea could supply a person with 12% of the recommended daily intake manganese. Consumption of 100-g of peas can also supply 5% phosphorus, 11% iron and 6% magnesium of the recommended daily intake (Makhlouf et al., 1995).

2.2.1.2.3 Fibre, carbohydrates, fat and other nutritional factors

Peas are good sources of fibre. Consumption of 100 g of peas can provide 10% of the recommended dietary fibre intake. Peas are predominantly carbohydrate, and 100 g pea contains 171.5 kJ, of which 125.5 calories comes from carbohydrate, 40 calories from protein and 6 calories from fat. The form of fat is omega-3 and omega-6 fatty acids (Alonso et al., 2000).

Peas also contain phytosterols, especially β -sitosterol. Makhlouf et al. (1995) reported that vegetables such as legumes and cereals, which are rich in plant sterols can help reduce cholesterol levels in the human body. Fresh peas also contain antioxidant flavonoids such as carotenes, lutein, and zeaxanthin (Igbasan & Guenter, 1996).

2.2.2 Pea protein

Pea protein is an important protein in leguminous plants due to its high protein content. The protein content of commonly grown pea cultivars ranges from 22% to 32% per 100 g, making it a significant protein source in human and animal nutrition (White, 1983). Protein concentrates and isolates have been commercially produced from dried peas for over 50 years (Owusu-Ansah & McCurdy, 1991). The main pea protein is globulin, which has minimal solubility at its isoelectric point region (pH 4.0~6.0) (Barac et al., 2010). The solubility of pea protein increases rapidly under neutral, alkaline or extreme acidity. The maximum solubility of close to 80% was observed in the pH range 8-9 (Fernández-Quintela et al., 1997). The denaturation temperatures of pea globulin vicilin (7 S) and pea globulin (11 S) have been reported as 83 °C and 92 °C, respectively (Barac et al., 2015).

2.2.2.1 Nutritional characteristics of pea protein

Unlike some plant-based protein powders, pea protein is hypoallergenic, highly bioavailable and well-digested (Schaafsma, 2000).

2.2.2.1.1 Well-balanced amino acid

Pea protein is valuable for human nutrition due to its well-balanced amino acid profile (Table 2.2), which is similar to soy. It is a "complete protein" containing all nine essential amino acids for humans (Tömösközi et al., 2001) and it is rich in branched-chain amino acids (BCAAs) (Tömösközi et al., 2001). When used as a supplement in food manufacturing, pea protein has the advantage of having lower allergenicity compared to soy and whey (Campos-Vega, Loarca-Piña, & Oomah, 2010).

Component	Parameter	Unit	per 100 g
Energy	Energy	kcal	364
	Protein	g	72.73
Proximate composition	Total lipid (fat)	g	6.06
	Carbohydrate, by difference	g	3.03
	Calcium, Ca	mg	61
Minerals	Iron, Fe	mg	19.09
	Sodium, Na	mg	1000
	Tryptophan	g	0.639
	Threonine	g	2.836
	Isoleucine	g	6.088
	Lysine	g	5.448
	Methionine	g	0.639
	Cystine	g	1.085
	Phenylalanine	g	4.006
	Tyrosine	g	2.712
Amino acids	Valine	g	3.585
	Arginine	g	6.152
	Histidine	g	1.788
	Alanine	g	2.952
	Aspartic acid	g	8.552
	Glutamic acid	g	12.988
	Glycine	g	2.988
	Proline	g	3.158
	Serine	g	3.782

Table 2.2 The nutritional characteristics of pea protein

Source: USDA Research Service Branded Food Products Database, Report: 45333760 (2018)

Pea protein is one of the richest dietary sources of lysine (82 g /kg protein) (Vermeirssen et al., 2003). Lysine is essential for building connective tissue such as skin, cartilage and bones and has been shown to help the absorption of calcium (Unni et al., 2012). Lysine has also been reported can reduce anxiety and help balance blood glucose (Smriga et al., 2004). Additionally, the pea protein contains more than three times the

amount of arginine per gram than whey protein (Weihai, 2017). Arginine (L-arginine) plays a key role in muscle growth, assisting in protein synthesis and increasing blood flow to muscles.

2.2.2.1.2 Hypoallergenic properties of pea protein

Pea protein is free of lactose, gluten, soy and nuts. It is naturally vegan and suitable for people with food allergies or with restricted dietary requirements (Swagerty, Walling, & Klein, 2002). Due to the lack of lactase, nearly two-thirds of the adult population in the world has some difficulty in digesting lactose (Vesa, Marteau, & Korpela, 2000). Gluten intolerance is the adverse reaction to gluten present in wheat, barley and rye. It is another common problem. The most severe symptom of intolerance to gluten is celiac disease (Jacob & Sotoudeh, 2002). Pea protein has the potential to help to alleviate the intolerance problems.

2.2.2.2 Isolation of pea protein

There are three forms of pea protein comprising of pea flour, pea protein concentration and pea protein isolate (Tömösközi et al., 2001). The average protein, starch and fat composition of pea flour, concentrate and isolate are shown in Table 2.3. Pea flour is made by the dehulling of the peas and grind it into powder. Pea protein concentrate can be prepared by corrosive filtration of the pea. The protein isolate is processed by wet preparation (dissolving the proteins in water, acid, or alkali) which increases protein content from 48% to 90% (Owusu-Ansah & McCurdy, 1991).

Composition	Whole seed /Flour (%)	Concentrate (%)	Isolate (%)
Protein	25	50	85
Starch	50	17	0
Fat	5-6	4	<3

Table 2.3 The average composition of pea flour, concentrate and isolate (O'Kane, 2004)

The main method used for the preparation of pea protein isolate involves the precipitation of the protein by isoelectric precipitation, followed by centrifugation and collection. First, the peas are ground into powder, followed by dissolving the proteins in water, acid, or alkali and then centrifuged to expel the insoluble materials. The precipitated protein curd is further centrifuged or sieved. The precipitate or curd is dried to produce protein isolate (Morita &

Kiriyama, 1993). The protein isolate can be also prepared by "salt extraction", "hydrophobication", and ultrafiltration (Murray, Pizzorno, & Pizzorno, 2005). Figure 2.3 shows a schematic process of the isoelectric precipitation process for the production of pea protein isolates (Boye, Zare, & Pletch, 2010).



Figure 2.3 Isoelectric precipitation process for the production of pea protein isolates (Boye, Zare, & Pletch, 2010)

Several factors affect the yield of protein isolate from the isoelectric precipitation method such as powder particle size, solubilizing medium and the pH of solubilisation (Nielsen, Petersen, & Dambmann, 2001). According to Scopes (2013), the best average particle size for this method is between 100 μ m to 150 μ m for protein solubilisation. Larger particle sizes lead to a lower protein yield. A similar yield is obtained when using potassium hydroxide and sodium hydroxide as the solubilising protein medium. However, when calcium hydroxide is used as the solubilizing medium, less than 10% pea protein dissolves because of the salting-out effect

of calcium ions (Arai, Nojiri, & Naito, 1998). Acids are also commonly used for solubilization, except for sulphuric acid, this is possibly due to the effect of precipitating of the sulfate ion lower than other acids. (Gueguen, 1980). The pH of the solubilizing and precipitating agents is another factor that affects the composition of the isolate. At less than pH 5.3, the protein isolate will have lower protein content and higher lipid content than at or above pH 5.3 (Fredrikson et al., 2001).

Salt extraction takes advantage of the salting-in and salting-out phenomena of proteins, followed by a desalting process to lower the ionic strength of the protein environment (Boye, Zare, & Pletch, 2010). Briefly, flour is stirred for 10–60 minutes in a salt solution of specified ionic strength at a 1:10 (w/v) ratio, followed by the removal of the insoluble matter by settling, decanting, screening, filtering, or centrifuging. The supernatant is then desalted and dried (Gueguen & Barbot, 1988). This method of salt extraction is convenient and straightforward and can be used for crude extraction and concentration of proteins. However, the protein concentration purified by salting out is lower and needs to be combined with other methods to gain high concentration refined protein (Murray, Pizzorno, & Pizzorno, 2005).

2.2.2.3 Health benefits of pea protein

Pea protein is almost as good as whey protein for building muscle and recovering after workouts (Tang et al., 2009). This leguminous protein can also help reduce blood pressure and decrease the risk of diabetes (Paddock, 2009).

Pea protein is rich in branched-chain amino acids (BCAAs) which build muscle (Schaafsma, 2000). BCAAs stimulate protein synthesis and account for more than 35% of muscle mass, and therefore, are ideal choices to rebuild and recover muscles after work-out.

Another advantage of pea protein is that it does not contain starch and fiber which can cause undesirable effects such as gas and bloating (Weigle et al., 2005). Pea protein is more comfortable on the stomach than dairy-based protein due to its highly digestible (94%) (Le Guen, 1995).

Pea protein does not raise blood sugar levels as do fruit juices or other high-carbohydrate foods due to the low concentration of sugar in peas. Besides, pea protein can assist the body in

spending more time digesting low glycemic food which can help to keep blood sugar even (Monro & Shaw, 2008). Low glycemic food will also slow down the rate of stomach emptying and keep a feeling of fullness for longer. Therefore, pea protein can help to maintain healthy body weight and avoid the risks associated with obesity and diabetes (Mollard et al., 2014).

2.2.2.4 Disadvantages of pea protein

Even though pea protein has many advantages, most products containing pea protein have a green colour and a bitter taste, making them mostly unacceptable to the customers. Also, pea protein has a lower solubility than dairy ingredients. Pea protein exhibits poor solubility in the low pH beverages, such as kefir and yogurt, even though it at low concentrations. Therefore, the applications of pea protein are low. Hence there is increased research interest in reducing the bitter flavour in pea ingredients and products. (Dahl, Foster, & Tyler, 2012).

2.2.2.5 Applications of pea protein

Pea protein can be found in a variety of products, from protein powders to meat to yogurt (Table 2.10). Based on the production procedures, pea protein can be divided into three groups: concentrates, with lower protein content which may contain some fat and carbohydrates; textured, which can be used as meat replacements; and isolates, which have the highest protein concentration (Campos-Vega, Loarca-Piña, & Oomah, 2010).

Between 2010 and 2014, four new types of products based on pea protein were produced: processed meat, fish, baked goods, snacks and desserts (Babault et al., 2015). Besides, between 2013 and 2014, food and beverage product promotions featuring pea protein expanded by 49% (Frost and Sullivan, 2012). Although less than 5 % of all protein items produced in 2012 utilised pea protein, globally non-soy class legume-based proteins were used in 34% of issues, with pea protein being present in 55% of the items in that vegetable class. Pea protein no two soy-problem highlights: allergens and GMO (genetically modified organism), so it possibly became the remarkable advance driver for pea protein. Consumer concerns about soy allergies and genetically modified soybean have motivated research into plant-protein alternatives. According to the Grand View Research market analysis (Grand View Research, 2017), the global pea protein market is expected to reach \$133.5 million in 2025, due to the high nutritional value and sustainability of the pea crop.

2.3 Beany flavour of pea protein

Beany flavour is an unpleasant flavour associated with pea products. Pea (*Pisum sativum*) as a source of high-quality protein presents economic and nutritional advantages for use in the animal and human food industry (Martinez et al., 2008). However, pea and pea proteins are not widely used in food applications because of their strong beany flavour (Klein & Raidl, 1986; Pattee et al., 1983). Undeniably, the application of pea as a healthful and low-cost source of protein for humans depends on the acceptance of consumers (Patil, 2017).

2.3.1 Volatile compounds contributing to the beany flavour

Numerous studies have attempted to identify and reduce the most potent odor-active volatiles in pea preparations (Jakobsen et al., 1998; Murray, Shipton & Whitfield, 1970; Murray et al., 1976). The volatiles of pea products include aldehydes, alcohols, ketones and furans (Kaneko, Kumazawa, & Nishimura, 2011; Shi et al., 2015; Suratman, Jeon, & Schmidt, 2004). A further study reported that typical beany flavour-causing compounds from pea products include pentanol, hexanol, heptanol, hexanal, and ethyl vinyl ketone (Wang et al., 1997). In the study of Suratman et al. (2004), hexanal was identified as the beany flavour-causing volatile compound that had the highest concentration among all the detected volatile chemicals in the studied pea protein. The similar high proportion of hexanal in the volatiles of raw pea was discovered by many studies (Yuan & Chang, 2007; Zhang, Guo, Liu, & Chang, 2012).

Table 2.4 shows the volatile compounds associated with beany flavour. The presence of hexanal has been reported in most published reports because it is considered one of the most important volatile compounds contributing to the beany flavour. However, many different compounds have been reported as beany flavour volatile compounds in published studies. This may imply that different researchers have dissimilar opinions on the chemical compounds that contribute to the beany flavour. It may also indicate that volatile compounds contributing to the beany flavour in one product may not be regarded as the major beany flavour compounds in another product.

Volatile beany flavour compound	Reference
Hexanal	Kaneko et al. (2011); Shi et al. (2015); Yuan and Chang (2007); Lei and Boatright (2008); Schindler et al. (2012)
Hexanal	Kaneko et al. (2011); Shi et al. (2015); Yuan and Chang (2007); Lei and Boatright (2008); Schindler et al. (2012)
1-Penten-3-ol	Suratman et al. (2004); Suratman et al. (2004); Yuan and Chang (2007)
1-Penten-3-ol	Suratman et al. (2004); Suratman et al. (2004); Yuan and Chang (2007)
Hexanol	Shi et al. (2015); Yuan and Chang (2007)
Hexanol	Shi et al. (2015); Yuan and Chang (2007)
1-Octen-3-one	Kaneko et al. (2011); Suratman et al. (2004); Schindler et al. (2012)
1-Octen-3-one	Kaneko et al. (2011); Suratman et al. (2004); Schindler et al. (2012)
1-Octen-3-ol	Kaneko et al. (2011); Shi et al. (2015); Schindler et al. (2012)
Trans, trans-2,4-decadienal	Shi et al. (2015); Yuan and Chang (2007)
Heptanal	Suratman et al. (2004)
n-Pentanal	Schindler et al. (2012)
2-nonenal	Suratman et al. (2004)
(E, Z)-2,4-decadienal	Kaneko et al. (2011)
Trans-2-hexenal	Shi et al. (2015)
Trans-2-nonenal	Yuan and Chang (2007)
Benzaldehyde	Suratman et al. (2004)
n-Hexan-1-ol	Schindler et al. (2012)
Guaiacol	Schindler et al. (2012)
Nonanal	Suratman et al. (2004)

Table 2.4 Beany flavour volatile compounds

2.3.2 Causes of beany flavour

Peas contain unsaturated lipids and beany flavours can be caused by lipid oxidation that occurs in the production of pea protein. Lipid oxidation can take place via both enzymatic and non-enzymatic routes which detailed in the next part (Sikorski & Kołakowska, 2010).

The basic mechanism of lipid oxidation is shown in Figure 2.4 (Reineccius, 2006). The process is a radical reaction which includes three steps: initiation, propagation and termination. As seen in Figure 2.4 a lipid acid radical is formed from an unsaturated lipid in the initiation stage. Due to the instability of the lipid acid radical, it reacts with oxygen to generate a lipid peroxyl radical which is also unstable. Consequently, it reacts with another unsaturated lipid to create a lipid hydroperpoxide. This process recurs until an antioxidant stops it.

An enzyme is known as lipoxygenase (LOX) involved in the lipid oxidation of pea foods. The initiation stage of the LOX pathway is different from the basic lipid oxidation because molecular oxygen is involved in catalysing the reaction (Sikorski & Kołakowska, 2010). Unsaturated lipid acids react to produce unstable lipid hydroperoxides which can further decompose to a variety of carbonyls such as aldehydes. These carbonyl compounds can generate beany flavours in pea protein. Figure 2.5 shows the mechanism of the formation of volatile compounds by the lipid peroxidation process (Ayala, Muñoz, & Argüelles, 2014). After lipid hydroperoxides are formed, they further decompose to form alcohols, aldehydes, ketones and furans which also contribute to the beany flavour in pea protein.



Figure 2.4 Formation of volatile compounds due to the breakdown of lipid hydroperoxides (Reineccius, 2006)





Figure 2.5 Lipid peroxidation process (Ayala, Muñoz, & Argüelles, 2014)

2.3.3 Reducing beany flavour in pea protein

Many methods have been reported to reduce the beany flavour of pea protein. Besides the physical, chemical and flavour masking methods, a reduction in beany flavour can also be achieved by fermentation.

2.3.3.1 Physical method for reducing beany flavour in pea protein

Many treatments have been reported to improve the flavour of the pea products by inactivating LOX because LOX is essential to the reaction of lipid oxidation which decomposes to form beany flavour volatile chemicals (Wolf, 1975).

Hot grinding of pea protein is an effective heat treatment for reducing LOX activity (Lv et al., 2011). Table 2.5 summaries the beany flavour volatile compounds reported being reduced by hot grinding in different studies. Hot grinding significantly reduced the LOX activity of raw soymilk and markedly lowered the production levels of odour compounds such as hexanal, hexanol and 2-pentylfuran which cause beany flavours (Zhang et al., 2012). Similar results were observed by Mizutani and Hashimoto (2004) who showed that 80 °C was an effective pea grinding temperature to reduce lipid hydroperoxide activity as well as beany flavour content from n-hexanal and 1-hexanol. Lv et al. (2011) reported that a grinding temperature of between 80 °C and 100 °C could effectively reduce beany flavour volatile compounds including n-hexanal, 1-hexanol and 1-octen-3-ol.

Table 2.5 Wajor beany havour volatile compounds reduced by not grinding				
Major beany flavour	Grinding	Reference		
volatile compounds	temperature (°C)			
Hexanal				
Hexanol				
2-Pentylfuran	80.5	Zhang et al. (2012)		
1-Octen-3-one				
1-Octen-3-ol				
n-Hexanal	90	Mizutani and Hashimoto (2004)		
1-Hexanol	80			
n-Hexanal				
1-Hexanol				
1-Octen-3-ol	80-100	Lv et al. (2011)		
Trans-2-hexenal				
Trans, trans-2,4-decadienal				

Table 2.5 Major beany flavour volatile compounds reduced by hot grinding

Besides hot grinding, ultra-high temperature (UHT) processing has been reported to be a successful method to reduce the beany flavour volatile compounds (Zhang et al., 2012). In theory, a higher temperature can accelerate the inactivation of LOX enzymes which can reduce the rate of lipid oxidation resulting in less beany flavour volatile chemicals.

Several studies used more than one heat treatment method to reduce the beany flavour, combining different heat treatment methods such as hot grinding and UHT processing (Mizutani & Hashimoto, 2004; Zhang et al., 2012; Kwok & Niranjan, 1995). The combination of different heat treatment methods appeared to be more effective in reducing beany flavours than a single treatment alone (Mizutani & Hashimoto, 2004).

2.3.3.2 Chemical methods

In addition to physical methods, various chemical processes have been investigated for their ability to reduce beany flavour in pea protein. Chemicals such as cyclodextrins and antioxidants can be used to reduce the pea beany flavour compounds by removing them after their formation (Suratman et al., 2004). Antioxidants such as Vitamin E and Vitamin C were also investigated for their effects on reducing the beany flavour in soybean (Dahuja & Madaan, 2004).

2.3.3.3 Flavour masking

Another solution to the undesirable beany flavour is masking or covering the beany flavour in pea products by the addition of flavours or other compounds. Beany flavours were reduced through the unfolding of the secondary structure of PPI by forming solid dispersions with gum arabic or maltodextrin during spray-drying (Lan et al., 2019). Also, beany flavour could be covered by blueberry flavour (Potter et al., 2007).

2.3.3.4 Fermentation

The undesirable off-flavour of pea-based products has limited the consumption of the products and the application of pea protein. Fermentation has been a traditional option to overcome these limitations to produce acceptable products to the consumer (Rivera & Gallardo, 2010). The characteristic acceptable aroma and flavour of fermented pea protein foods are partially attributed to the metabolism of lactic acid bacteria (LAB) (Li et al., 2014).

Much of the current literature agrees that fermentation can reduce the beany flavour of legume protein (Blagden & Gilliland, 2005; Moy, Lu, and Chou, 2012; Schindler et al., 2012), and this is commonly attributed to the change or decrease of volatile compounds in the protein extract (Kaneko, Kumazawa, & Nishimura, 2011; Shi et al., 2015; Suratman, Jeon, & Schmidt, 2004). Hexanal is assumed to be the principal compound contributing to the beany flavour by many studies (Yuan & Chang, 2007; Zhang, Guo, Liu, & Chang, 2012).

The application of fermentation to remove the beany flavour has been reported in several studies (Table 2.6). Table 2.6 lists the major beany flavour volatile compounds reduced by fermentation. Blagden and Gilliland (2005) reported that both *Streptococcus thermophilus* and *Lactobacillus acidophilus* could completely remove hexanal which is considered a major beany flavour compound in soymilk. Other volatile compounds, such as methanol and acetaldehyde were also reduced compared to the control sample. This finding was supported by another study showing that the beany flavour volatile compounds n-hexanal and n-hexanol in pea protein extracts could be reduced by lactic acid bacteria (*Lactobacillus plantarum* and *Pediococcus pentosaceus*) fermentation indicating that fermentation could either reduce the generation of beany flavour or mask the unpleasant beany flavour (Schindler et al., 2012). Moy, Lu, and Chou (2012) also found that the hexanal content from the volatile compounds in tofu (tofu fermented by *Aspergillus oryzae*) was significantly reduced compared with that in non-fermented tofu. These studies provide clear evidence that fermentation may have a positive effect on improving the flavour of pea products by reducing or eliminating the beany flavour volatile compounds.

Major beany flavour volatile compounds reduced	Bacteria for fermentation	Products	Reference
Hexanal Methanol Acetaldehyde	Streptococcus thermophilus and Lactobacillus acidophilus	Pea milk	Blagden and Gilliland (2005)
n-Hexanal n-Hexanol	Lactobacillus plantarum and Pediococcus pentosaceus	Pea Protein Extracts	Schindler et al. (2012)
n-Hexanal Heptanal Nonanal	Aspergillus oryzae	Sufu	Moy et al. (2012)

Table 2.6 Major beany flavour volatile compounds reduce by fermentation

2.4 Coconut milk

Coconut (*Cocos nucifera*), which belongs to the Palm family (*Arecaceae*), is grown in abundance in Malaysia, Polynesia and southern Asia. Coconut milk is a white, milky substance extracted from the white flesh inside the mature brown coconut (Figure 2.6). Coconut milk has a rich, creamy texture, and it is used in many traditional cuisines around the world (Belewu & Belewu, 2007). The use of the fruit often depends on its processing technique, or specifically the method of squeezing and thinning the coconut cream. Thicker products are mostly used for rich desserts, while thinner milk is used for soups and curries, and the thin, fluid milk is mostly used as a dairy-free milk substitute (Seow & Gwee, 1997).



Figure 2.6 The image of coconut (a) and coconut milk (b).

Note: Source of the figures-Google image

2.4.1 Nutritional characteristics of coconut milk

Coconut milk contains high levels of saturated fat, making it a very high-calorie food. About 93% of its calories come from fat, including saturated fats (Pehowich, Gomes, & Barnes, 2000). Coconut milk is also rich in vitamins and minerals, although the nutritional content often differs by product (Papamandjaris, 1999). For example, coconut milk drinks, have a different nutritional profile to canned coconut milk. The carbohydrate, fat and protein content of 240g raw coconut milk and canned coconut milk are 13.3 g, 57.2 g, 5.5 g and 6.8 g, 51 g, 4.8 g, respectively (Seow & Gwee, 1997).

2.4.2 Healthy benefits of coconut milk

Previous studies suggest that coconut milk confers numerous health benefits such as stimulating weight loss (Dayrit, 2015), improving heart health (Khaw et al., 2018) and enhancing the immune system (Lappano et al., 2017).

Coconut milk contains medium-chain triglycerides (MCTs), which researchers have linked with increase metabolism, benefits body composition and help you lose belly fat (Dayrit, 2015). Coconut milk can significantly increase levels of high-density lipoprotein cholesterol (HDL), which protects the heart and removes low-density lipoprotein cholesterol (LDL) from the blood (Khaw et al., 2018). Coconuts contain significant amounts of fat, but unlike other nuts, they provide fat that is mostly in the form of medium chain saturated fatty acids (MCFAs) in particular, one called lauric acid (Dayrit, 2015). Lauric acid is converted in the body into a highly beneficial compound called monolaurin. Monolaurin is an antibacterial agent, an antiviral and antifungal, used to treat viral infections, including influenza (Elmore et al., 2014). It ultimately can boost the immune system and further fight against bacteria and yeasts. Therefore, it thought that consumption of coconut milk and other coconut-derived foods may help protect the body from infections and viruses (Lappano et al., 2017). Due to these benefits, coconut milk has gained popularity in the healthcare industry as an alternative to dairy milk.

2.5 Lactic acid bacteria

Lactic acid bacteria (LAB) are gram-positive, non-spore forming cocci, coccobacilli or rods. Although many genera of bacteria produce lactic acid as a primary or secondary end-product of fermentation, the term lactic acid bacteria are conventionally reserved for the order *Lactobacillales*, which includes *Lactobacillus*, *Leuconostoc*, *Pediococcus*, *Lactococcus* and *Streptococcus* (Klaenhammer, 1993).

LAB ferment glucose primarily to lactic acid, or to lactic acid, CO₂ and ethanol. Most of LAB grow anaerobically, but unlike most anaerobes, they grow in the presence of oxygen as "aerotolerant anaerobes". Although they lack catalase, LAB has superoxide dismutase and have alternative means to detoxify peroxide radicals, generally through peroxidase enzymes (Makarova et al., 2006).

Since LAB obtain energy only from the metabolism of sugars, they are restricted to environments in which sugars are present (Carr, Chill, & Maida, 2002). The microorganisms have limited biosynthetic ability, having evolved in environments that are rich in amino acids, vitamins, purines and pyrimidines, so they must be cultivated in complex media that fulfill all their nutritional requirements (Axelsson, 2004). Most of LAB are free-living or live in beneficial or harmless associations with animals and are commonly found in milk, milk products and decaying plant materials. In humans, they are normal flora of the oral cavity, the intestinal tract and the vagina, where they play a beneficial role in maintaining human health (Naidu, Bidlack, & Clemens, 1999).

Lactic acid bacteria are among the most important groups of microorganisms used in food fermentations performing an essential role in the preservation and production of wholesome foods (Carr, Chill, & Maida, 2002). LAB microorganisms contribute to the taste and texture of fermented products and inhibit food spoilage bacteria by producing growth-inhibiting substances and large amounts of lactic acid (Leroy & De Vuyst, 2004). LAB is used in the manufacture of dairy products such as acidophilus milk, yogurt, buttermilk, sour cream, and cheeses. LAB is also important commercially in the processing of meats (sausage, cured hams), alcoholic beverages (beer, wine), and vegetables (kimchi and sauerkraut) (Axelsson, 2004).

2.5.1 The application of lactic acid bacteria

Common lactic acid bacteria used as starter cultures include species of *Lactobacillus* (*Lactobacillus acidophilus*, *L. plantarum*, *L. reuteri*, *L. johnsonii*, *L. rhamnosus*, *L. casei*) and *Bifidobacterium* species (*Bifidobacterium. lactis*, *B. breve*, and *B. longum*) (Wang et al., 2010). In the dairy industry, the integration of LAB strains in traditional food products has resulted in the production of novel fermented dairy products: such as yoghurt, kefir and probiotic beverage (Chiang & Pan, 2012, Liong, Easa, Lim, & Kang, 2009). Typical lactic acid bacteria used in fermented foods and beverages are shown in Table 2.7.

In addition to dairy products, lactic acid bacteria (LAB) are also broadly used in plant food fermentations. This process can improve the nutritional quality and health benefits of plant foods, which has generated scientific research. Since consumers treat edible peas as important food, fermenting peas and pea products with LAB can improve their biological activity and nutritional composition (Weinberg et al., 1993).
Pea source	Starter culture	Temperature (°C)	Time (h)	Reference
Dried seeds of	Lactobacillus casei ATCC393, Lb. zeae LMG17315, Lb. paracasei BGHN14, Lb. rhamnosus BGT10,			
green pea	Lb. plantarum LMG9208, Lb. plantarum BGBUK2-5, Lb.plantarum PV2-45a, Lb. plantarum BGGA8, Lb. plantarum BGH010.	30	16	Stanisavljević et al., 2015
"Opal" pea	Lactobacillus plantarum	30	18	Czarnecka et al., 1998
Cow milk and pea milk mixtures. (Pea protein isolate: Nutralys [®] S85F)	lyophilized S. thermophilus, Lactobacillus bulgaricus, S. thermophilus 102303T, L. delbrueckii subsp. bulgaricus 104365, L. acidophilus 76.13, L. helveticus CNRZ 303, Lactobacillus casei subsp. casei ATC 334, Lactobacillus rhamnosus CRBIP 24.130, Lactobacillus fermentum	37	24	Yousseef et al., 2016
	CRBIP 24.11			

Table 2.7 Lactic acid bacteria used as starter cultures for pea source fermentation

Stanisavljević et al. (2015) selected nine LAB strains (Table 2.7) to test their ability to hydrolyse pea proteins and tested their ability to grow in pea seed protein-based media. Two strains, Lactobacillus rhamnosus BGT10 and Lactobacillus zeae LMG17315, displayed strong proteolytic activity for pea proteins and significantly increased the antioxidant activity of pea seed-based medium during fermentation. Yousseef et al. (2016) sellected five mixtures of milk and pea protein (Volum ratio: 100:0, 90:10, 80:20, 70:30, 60:40 respectively) and fermented them at 37 °C for 24 h by ten starter cultures (show in Table 2.7) of lactic acid bacteria (10⁷) CFU /mL) to select the concoctions which can product yoghurt that similar to a conventional yoghurt. The result showed that two groups of products of 0 g or 10% pea protein seemed to be the most similar to traditional dairy products. The third group (20% pea protein) included products fermented with two starters: Streptococcus thermophilus and Lactobacillus helveticus with negative characteristics such as astringency and bitterness. four starter cultures: Streptococcus thermophilus + Lactobacillus delbrueckii subsp. bulgaricus, Streptococcus thermophilus + Lactobacillus delbrueckii subsp. bulgaricus, Streptococcus thermophilus + Lactobacillus acidophilus and Streptococcus thermophilus + Lactobacillus casei subsp. casei seem promising for the fermentation of milk and pea protein mixtures.

2.5.2 Bifidobacterium

Non-motile *bifidobacteria* with variable morphology are anaerobic gram-positive bacteria. They are a major (92%) group of gastrointestinal tract microbiota of mammals. *Bifidobacterium* degrades carbohydrates through a particular fructose-6-phosphate phosphoketolase pathway, known as the "bifid shunt" (De Vuyst et al., 2014), where the fructose-6-phosphoketolase enzyme (EC 4.1.2.2) plays a key role (Figure 2.7) (Killer et al., 2010). Additional enzymes are needed to channel various diet- and host-derived carbon sources into the bifid shunt (Figure 2.7), which allows *bifidobacteria* to produce more energy in the form of ATP from carbohydrates than the fermentative pathways (Davidson & Chen, 2004).



Notes: *AckA*, Acetate kinase; *Adh2*, aldehyde-alcohol dehydrogenase 2; *Aga*, α-galactosidase; *Agl*, α-glucosidase; *AraA*, 1-arabinose isomerase; *AraB*, Ribulokinase; *AtsA*, sulfatase; *Bgl*, β-glucosidase; *Eno*, enolase; *GalE1*, UDP-glucose 4-epimerase; *GalA*, β-endogalactanase; *GalG*, β-galactosidase; *GalK*, galactokinase; *GalM*, glactosemutarotase; *GAPDH*, glyceraldehyde-3-phosphate dehydrogenase; *GlkA*, glucokinase; *Gpi*, glucose 6-phosphate isomerase; *Gpm*, phosphoglycerate mutase; *FrK*, frucktokinase; F6PPK, fructose-6-phosphoketolase; *FucI*, 1-fucose isomerase; *FucK*, 1-fuculose kinase; *FucA*, 1-fuculose-1-phosphate aldose; *FucO*, lactaldehyde reductase; *Ldh2*, lactate dehydrogenase; *LNBP*, lacto-*N*-biose phosphorylase; *NagA*, *N*-acetylglucosamine-6-phosphate deacetylase; *NagB*, glucosamine-6-phosphate deaminase; *NagK*, *N*-acetylglucosamine kinase; *Pst*, formate acetyltransferase; *Pi*, phosphote; *Pyk*, pyruvate kinase; *Rk*, ribokinase; *R5PI*, ribose-5-phosphate isomerase; *Tul*, transaldase; *Tkt*, transketolase; *UgpA*, UTP-glucose-1-phosphate uridylyltransferase; *XylA*, xylose isomerase; *XylB*, xylulose kinase

Figure 2.7 The fructose-6-phosphate phosphoketolase pathway in bifidobacterial (Pokusaeva, Fitzgerald & van Sinderen, 2011).

The metabolism of *bifidobacteria* has focused on oligosaccharide (metabolism), as these carbohydrates are available in their nutrient-limited environments. These are generally classified as plant-derived fructo-oligosaccharides or dairy-derived galacto-oligosaccharides (Mayo & Van Sinderen, 2010). Typical examples of the sugars are ribose, galactose, fructose, glucose, sucrose, maltose, melibiose and raffinose.

2.5.3 Plant-based fermented beverages

Due to the health-promoting properties of plant-fermented foods and beverages, these products are becoming increasingly popular among producers and consumers. The most popular and commercial plant-based fermented beverages are fermented tea, plant-based kefir and soybean yogurt (Table 2.8).

Product	Country	Microorganism(s)	Substrate
Tiouuci	Country	Saccharomycas caravisiaa other yeasts lactic acid	Wheat rye other
Bread	International	bacteria (LAB)	grains
Cheese	International	LAB (Lactobacillus lactis, Streptococcus thermophilus, Lb. shermanii, Lb. bulgaricus), Propionibacterium shermanii, sometimes moulds (Penicillium spp.)	Milk
Fufu	West Africa	LAB, <i>Citrobacter freundii</i> , <i>Geotrichum</i> sp., <i>Candida</i> sp. and <i>Saccharomyces</i> sp.	Cassava root
Gari	West Africa	Corynebacterium manihot, yeasts, LAB (Lb. plantarum, Streptococcus spp.)	Cassava root
Idli	Southern India	LAB (Leuconostoc mesenteroides, Enterococcus faecalis), Torulopsis, Candida, Trichosporon pullulans	Rice and black gram
Kefir	North Africa	LAB	Milk
Kenkey	Ghana	LAB (pediococcus cerevisiae, Leuconostoc mesenteroides, and Lc. fermentum)	Maize
Kimchi	Korea	LAB	Cabbage, vegetables, sometimes seafood, nuts
Nan	India	Saccharomyces cerevisiae, LAB	White wheat flour
Ogi	Nigeria, West Africa	Lactic bacteria Cephalosporium, Fusarium, Aspergillus, Penicillium spp., Saccharomyces cerevisiae, Candida mycoderma, C. valida, or C. vini	Maize
Olives	Mediterranean	Lc. Mesenteroides, Lb. plantarum	Green olives
Pickles	International	Pediococcus cerevisiae, Lb. plantarum	Cucumber
Plara	Thailand	Bacillus sp., Bacillus cerus, B. circulans, B. licheniformis, B. megaterium, B. pumilus and B. subtitils	Fresh water and marine fish

Table 2.8 Typical commercial fermented products (Ray & Joshi, 2014)

2.6 Physical-chemical, nutrition, microbiological and sensory characteristics of plantbased fermented probiotic beverages

2.6.1 Acidity

The acidity of the medium during fermentation is a key indicator of the metabolic activity of microorganisms under certain environmental conditions and is an important fermentation parameter (Hwang et al., 2004; Lee, Miyahara, & Noike, 2002). The acidity has a promote function on the growth of bacteria and the accumulation of products, therefore, it is necessary to monitor the pH during fermentation. Although most microorganisms can grow in the pH range of 3-4 the pH must be kept constant over a narrow range to achieve high growth rates and optimal product formation (Calsamiglia, Ferret, & Devant, 2002). The accumulation of organic acids increases the titratable acidity decreases the pH of the products (Freire, Ramos, & Schwan, 2015, Akin & Ozcan, 2017 and Menezes et al., 2018).

2.6.2 Viable cell counts

There are different types of probiotic bacteria used in plant-based fermented beverages, and their growth rates vary with the fermentation substrate and environment. Generally, probiotic bacteria grow rapidly under optimum fermentation conditions resulting in high viable cell counts after fermentation. Cell counts may change during storage of the fermented products depending on several factors including the presence of residual sugars, organic acids produced, buffering capacity of the medium, product composition, fermenting microorganisms, storage conductions and packaging.

Ertanto et al. (2009) reported cell counts in coconut yogurt ranging from 7 to 9 Log CFU /mL During fermentation of soymilk to reduce the beany flavour, Telang et al. (2010) reported cell counts of 10⁹ CFU /mL after 12 h of fermentation. Also, acceptable fermented soymilk supplemented with skimmed milk powder at 5% level with similar levels of viable cell counts have been reported (Telang et al., 2010). Commercial probiotic, *Lactobacillus paracasei* LBC-81, was used singly and in co-culture with potential probiotic yeasts, *Saccharomyces cerevisiae* CCMA 0731, *S. cerevisiae* CCMA 0732, and *Pichia (P.) kluyveri* CCMA 0615, to ferment a maize-based substrate (Menezes et al., 2018). All the tested strains presented viability higher than 6 log CFU /mL, as recommended for food probiotic products with the exception of the yeast *P. kluyveri* which decreased during fermentation and storage.

Similar results were reported during the fermentation of LAB and yeasts in cassava (Freire, Ramos, & Schwan (2015). There was a significant (p<0.05) growth of LAB (8 log CFU /mL) during the first 6 h. However, after 12 h, there was a significant (p<0.05) reduction of LAB when co-cultivated with the yeasts *S. cerevisiae* CCMA 0232 (7.6 log CFU /mL), *T. delbrueckii* CCMA 0234 (7.8 log CFU /mL), and *P. caribbica* CCMA 0198 (7.7 log CFU /mL). After 24 h of fermentation, the LAB population had increased to around 8 log CFU /mL.

2.6.3 Colour

The colour of fermented protein beverages changes depending on the substance of fermentation. Akin & Ozcan (2017) studied changes in the colour of fermented milk containing plant proteins during storage. The plant proteins included rice protein, wheat gluten, soy protein isolate and pea protein isolate. There were no marked differences in the L*value (lightness) of various beverages. They reported that the fermented milk beverage containing soy protein isolate recorded the highest L*value change while the fermented beverage of rice protein had the highest b* value (yellowness) due to the yellow rice protein. When *Streptococcus thermophilus* and *Lactobacillus bulgaricus* were used to ferment soy flour cowpea and peanut seed extract powders, the initial colour was similar with that of commercial buttermilk products (Schaffner & Beuchat, 1986). The b* value of soybean powder was the highest, while the cowpea and peanut powders displayed a similar degree of yellowness compared to the cowpea milk powder. In summary, little difference was distinguished between the colour changes of soybean, cowpea and peanut induced by *L. bulgaricus* and *S. thermophiles* (Schaffner & Beuchat, 1986).

2.6.4 Sugar

The sugar concentration may change during both the fermentation of plant-based beverages and their storage (Corona et al., 2016). Czarnecka et al. (1998) researched the effect of fermentable sugar levels on the dynamics of lactic fermentation during the fermentation of milled legume seeds and plant materials with *Lactobacillus plantarum*. They found that after the fermentation and extrusion, the levels of verbascose, stachyose and raffinose were significantly reduced by 80-90%. Czarnecka et al. (1998) have a similar found that lactose is readily metabolised to galactose and glucose by some *Kluyveromyces* and *Streptococcus* strains (Czarnecka et al., 1998). Magalhães et al. (2011) studied the chemical composition and

microbial ecology of the yogurt drink and found that the lactose concentration decreased within the 24 hours of fermentation, ranging from 45 to 35 mg /mL. Irigoyen (2005) reached a similar conclusion, with kefir fermented by kefir grains.

2.6.5 Nutritional characteristics

The nutritional composition of plant fermented foods has been widely reported (Canibe, Virtanen, & Jensen, 2007; Mbugua, 1987; Shekib, 1994; Akin & Ozcan, 2017; Magalhães et al., 2011). In general, the original nutrients present in foods and beverages are preserved during fermentation, and in many instances, the nutritional value of the food is increased (Canibe, Virtanen, & Jensen, 2007). For example, the partial hydrolysis of some proteins, which occurs during fermentation makes the food easier to digest and be absorbed when consumed. LAB can also produce vitamins such as vitamin C and B, which are necessary for the human body. Also, after fermentation, minerals such as calcium in the food do not change, but the lactic acid produced after fermentation effectively increases the utilization rate of calcium and phosphorus in the human body (Mbugua, 1987).

There is limited information on pea protein fermentation and its functional properties due to the low solubility of pea protein relative to the low pH environment (pH 4-5). Shekib (1994), investigated changes in the nutritional properties (non-protein nitrogen, crude and true protein, amino acids) of lentils and chickpeas during natural fermentation. This research showed that non-protein nitrogen increased significantly (p<0.01) in the fermented products, whereas the crude protein and true protein unchanged. Czarnecka et al. (1998) analysed viable cell counts in milled legume seeds fermented with *Lactobacillus plantarum* strains. Lactic fermentation and extrusion of bean and pea seeds increased (p<0.05) the "in vitro" protein and starch digestibility.

Akin & Ozcan (2017) investigated the storage-related changes in the physicochemical and sensory properties of non-fat fermented milk drinks that contained soy protein isolate, pea protein isolate, wheat gluten and rice protein. During storage, the protein content increased in fermented milk beverage. They also found rice protein and milk fermented beverage had the lowest total protein content, while fermented milk beverages with pea protein isolate had the highest protein concentration. Also, Magalhães et al. (2011) studied the microbial ecology and chemical composition of kefir beverage fermented for 24 hours. In their study, the protein

content increased from 2.12% to 3.19%, while fat decreased from 3.63% to 2.34%, and the calcium increased slightly from 0.21 mg /mL to 0.22 mg /mL.

2.6.6 Sensory characteristics

The consumer acceptability of foods is most directly expressed by their sensory profile. Thus, foods can be characterised by several factors which include colour, flavour, texture, smell, odour and taste (Dominy, 2004). Food sensory analysis is a test method that obtains objective results by sensory evaluation of various properties of foods based on human perception, followed by statistical analysis of the data (Favaro Trindade et al., 2001). Feng et al. (2013) investigated variations in sensory characteristics through the evaluation of the aroma of fermented soy sauce koji. The sensory analysis showed an obvious increase in "musty" and "soy sauce-like" odours, whereas the beany attribute diminished significantly throughout koji fermentation. Besides, Menezes et al. (2018) studied the sensory properties of a fermented maize-based substrate with the commercial probiotic *Lactobacillus paracasei* LBC-81 using the 1-9 points hedonic scale. The overall mean sensory scores for acceptance of the fermented koji increased from 5.07 to 5.45 (p<0.05). In a similar study, Puerari, Magalhães and Schwan (2012) reported a higher mean acceptance sensory score (7.8) of the fermented cocoa kefir beverage than the research of Menezes et al. (2018).

3. MATERIALS AND METHODS

3.1 Introduction

The main aim of this research was two-fold, to reduce the yellowness of pea protein powder and then reduce the beany-flavour by chemical purification, and then add the purified paste into coconut milk for lactic acid fermentation. The study was conducted in three integrated phases. All the experiments were conducted in triplicate and all analyses were either duplicated or triplicated.

The first phase used the isoelectric precipitation and salt extraction methods to purify the commercial pea protein powder (Schindler et al., 2012). The purpose of this step was to reduce the yellowness of the pea protein powder to a creamy colour protein paste more suitable for wider applications including the production of the fermented beverage. The colour and protein content of the purified samples (pea protein paste) were compared with the original commercial pea protein powder. The second phase investigated the best fermentation conditions of fermented pea protein-coconut milk beverage (optimization). The fermentation mixture of refined pea protein paste and organic coconut milk (Ceres Organics, NZ) was fermented by a mixed lactic acid bacteria culture. The fermentation was conducted in two parts. The first part comprised a single-factor test which investigated the effect of three factors (temperature, time and pea protein paste concentration). The second part used the orthogonal test to investigate the optimum fermentation conditions for the fermented pea protein-coconut milk beverage. During fermentation, pH, titratable acidity (TA), viable cell counts (VCCs), colour and protein content were determined. In the orthogonal test, supplementary information was obtained through sensory evaluation of the fermented beverage using a semi-trained sensory panel (n=18). The orthogonal test is intended to screen optimum conditions using a reduced number of experiments (Oztop, Sahin, & Sumnu, 2007).

The main purpose of phase two was to reduce the beany flavour of the pea protein through fermentation to develop a new fermented pea protein-coconut milk beverage. In phase three, the stability of the fermented pea protein coconut beverage, which included the sensory characteristics were determined during storage (4 °C) for 21 days. An overview of the three phases used in this study is shown in Figure 3.1



Figure 3.1 Experimental phases of the study



3.2 Description of materials

A dark yellow commercial pea protein powder (Roquette S85F, France) was supplied by White Rock Foods Limited (Auckland, New Zealand). The starter culture VEGE 053 LYO (Danisco, USA) which is recommended for vegetable and plant fermentations was supplied by Dupont[®] Limited (Auckland, New Zealand). The culture contained *Streptococcus thermophilus*, *Lactobacillus delbrueckii* subsp. *bulgaricus*, *Lactobacillus delbrueckii* subsp. *lactis*, *Bifidobacterium lactis* (HN019TM) and *Lactobacillus acidophilus* (NCFM[®]). A dosage of 10-20 DCU /100 L was used as per supplier recommendations. Organic coconut milk (Ceres Organics, NZ) was purchased from a local supermarket in Auckland, New Zealand.

3.3 Phase 1: Refining of commercial pea protein powder

3.3.1 Description of the refining method

The isoelectric precipitation and salt extraction methods were used to purify the commercial protein powder with minor modifications (Schindler et al., 2012). The protein powder (100 g) was suspended in about 90 mL distilled water and then made up to 1 L to give a 10% solution. The mixture was heated in a water bath (Grant, Global Science, NZ) with gentle mixing using a vortex mixer (Dalson, Dalsonware Pty. Ltd., Australia) until the temperature had stabilised at 60 °C which is ideal for the protein extraction (Kizer, Renninger, & Stiles, 2019). The pH was adjusted to 9 using 1 M NaOH (Univar, Ajax Finechem Pty Ltd, NZ) and the solution was mixed using a vortex mixer (Dalson, Dalsonware Pty. Ltd., Australia) for 10 minutes. The extract was separated by centrifuging (SorvallTM LegendTM X1, Fisher Scientific, NZ) at 16211 g for 10 minutes. The supernatant was removed and further centrifuged to remove any remaining precipitation. Ten (10) g calcium chloride (4 M, CaCl₂) (Sigma-Aldrich Corporation, USA) were added to the supernatant. The mixture was adjusted to pH 5 with 1 M hydrochloric acid (HCl) (Sigma-Aldrich Corporation, USA), followed by centrifugation for 10 minutes at 16211 g. The pea protein has the lowest solubility at pH 5 (Taherian et al., 2011); The isoelectric point of pea protein lies between pH 4 and pH 6. The supernatant was discarded and the pea protein paste (pellet) was collected. The paste was re-suspended in water (30 x dilution by weight) and then mixed for 1 minute using a homogenizer (Thomas Scientific LLC, USA). The sample was further centrifuged (16211 g) for 5 minutes and the protein paste was collected. An overview of the refining process is shown in Figure 3.2.



Figure 3.2 Modified commercial pea protein powder refining procedure (Schindler ey al., 2012)

Notes: CPPP = commercial pea protein powder; Photos captured by iPhone XR, Apple Inc., California, USA.

3.3.2 Measurement of colour

The colour of the commercial pea protein powder and refined protein isolate obtained from the commercial pea protein powder were measured using the Konica Minolta spectrophotometer (CM-5, Japan) following the method of Kurtmann et al. (2009). According to the L*, a*, b* colour system, L* represents lightness (0 is black and 100 is diffuse white), a* is the green /red scale (negative values represent green, positive values are red) and b* is the blue /yellow scale (negative values indicate blue, positive values are yellow) (Alqahtani, Aljurais, & Alshaafi, 2012). Before the measurement, the spectrophotometer was allowed to warm up for one minute. Commercial pea protein powder and refined protein isolate (0.3 g) were mixed in three (3) mL water then transferred into four (4) mL plastic cuvettes (Sigma Aldrich, NZ) respectively and the colour of the samples were measured using the spectrophotometer.

3.4 Phase 2: Selection optimum fermentation conditions of fermented pea proteincoconut milk beverage

3.4.1 Single factor test design

The effects of different fermentation temperatures, protein concentrations and fermentation times on the mixture of refined pea protein paste and organic coconut milk (Ceres Organics, NZ) were investigated using the single factor test.

3.4.1.1 Effect of fermentation temperature on the fermentation of pea protein-coconut milk beverage

According to previous studies (Leroy & De Vuyst, 2004; Yildiz 2009), the optimum fermentation temperatures for *S. thermophilus*, *L. delbrueckii* subsp. *bulgaricus*, *L. delbrueckii* subsp. *lactis*, *B. lactis* and *L. acidophilus* in (fermented) beverages range between 35 °C and 45 °C with pH ranging from 3.5 and 4.5. In these studies, the fermenting bacteria were inoculated either singly or mixed. In our study, the selected fermentation temperatures (fixed factors) were 35 °C, 37 °C, 40 °C, 43 °C and 45 °C with final pH between 3.5 and 4.5.

Experiment	Fermentation temperature (°C)
1	35
2	37
3	40
4	43
5	45

Table 3.1 Effect of temperature on the fermentation of 100 mL coconut milk for 12 h

For the experiments shown in Table 3.1, 5×100 mL organic coconut milk (Ceres Organics, NZ) samples were pasteurized at 95 °C in a water bath (Grant, Global Science, NZ) for 15 minutes and then cooled to 30 °C. The temperature was monitored using a thermocouple (Fluke 51, Fisher Scientific, NZ). When the temperature of the coconut milk had stabilised at 30 ± 1 °C, the starter culture (VEGE 053 LYO) was directly inoculated into the coconut milk at a dosage of 20 DCU /100 L (0.014 g /L). The mixture was agitated for about 10 minutes using a vortex mixer (Dalson, Dalsonware Pty. Ltd., Australia) at low speed to avoid foaming and incorporation of air. The inoculated coconut milk was allowed to ferment for 12 h in the respective water baths set at 35 °C, 37 °C, 40 °C, 43 °C and 45 °C. pH was measured directly every 2 h by a digital pH meter (Sartorius PB-20, USA) equipped with a glass electrode (AOAC 981.12, 2005). The pH meter was calibrated with standard buffer solutions (LabServ, Thermofisher, NZ) at pH 4.0 and 7.0 before measurement.

3.4.1.2 Effect of refined pea protein concentration on the fermentation of pea proteincoconut milk beverage

The protein concentration of fermented beverages in the market ranges from 3 to 12% (Corbo et al., 2014; Özer & Kirmaci, 2010). The fermented products include ginger beer, milk /water kefir and kombucha. To study the effect of pea protein concentration on the fermentation of fermented pea protein-coconut milk beverage, five refined pea protein paste concentrations were used (Table 3.2) with 45 °C (result from section 3.4.1.1) as the fermentation temperature.

Experiment	Refined pea protein (%)		
1	3		
2	5		
3	7		
4	9		
5	11		

Table 3.2 Concentrations of refined pea protein paste (%) added to 100 mL coconut milk for fermentation at 45 °C for 12 h

 5×100 mL organic coconut milk samples were pasteurised and cooled as previously described (section 3.4.1.1). When the temperature of the coconut milk had stabilised to $30\pm^{\circ}$ C, variable amounts of refined pea protein paste (Table 3.2) were added to the respective milk samples and then mixed (Dalson, Dalsonware Pty. Ltd., Australia). The starter culture (VEGE 053 LYO) was directly inoculated into the five treatments of refined pea protein paste- coconut milk mixtures at the same dosage used in section 3.4.1.1, and then mixed as previously described. The mixtures were fermented at 45 °C in a water bath for 12 h, with 2-hourly measurements of pH as previously described (section 3.4.1.1).

3.4.1.3 Effect of time on the fermentation of pea protein-coconut milk beverage

The starter culture VEGE 053 LYO contains several LABs which are representative members of commercial probiotics. Although the species of bacteria in the culture are not designated as probiotics, their presence in the final fermented product may be beneficial (Marsh et al., 2014). FAO /WHO states that to exert a beneficial effect to human health, the probiotics must be alive and available in high numbers, at least 10^6 colonies forming per unit (CFU) at the time of consumption (2002). To select the optimum fermentation time for the fermented pea protein-coconut milk beverage, the experiment was set up as described in section 3.4.1.1, but the fermentation was allowed to continue for 24 h at 45 °C, 3% pea protein concentration (results from section 3.4.1.1 and 3.4.1.2). The samples were collected every 2 h and stored At -18 °C (Fisher & Paykel, NZ) for the enumeration of viable cell counts (VCCs).

3.4.1.4 Enumeration of viable cell counts (VCCs)

Total viable cell counts of the beverage during fermentation were determined by pour plating diluted samples on De Man, Rogosa and Sharpe (MRS) agar and M17 agar (Oxoid, UK). One (1) mL of each sample was mixed with 9 mL sterile peptone water (Merck, Germany), and then

serial dilutions (10^1 to 10^8) were prepared and plated using the pour plate method (Jackson et al., 2000). The MRS agar plates were used to enumerate *L. delbrueckii* subsp. *bulgaricus*, *L. delbrueckii* subsp. *lactis*, *B. lactis* (HN019TM) and *L. acidophilus* (NCFM®), whereas M17 agar (Oxoid, UK) was used to enumerate *S. thermophilus*. The solidified MRS agar plates were incubated at 37 °C for 72 h under anaerobic conditions using an Anaergen park (AN0035A) (Mitsubishi Gas Chemical Company Inc., Japan) and 48 h for M17 agar plates at 37 °C under aerobic conditions. Results of the enumerated cell counts were expressed as log colony forming units per milliliter (CFU /mL).

3.4.2 Orthogonal test

3.4.2.1 Description of the orthogonal test design

Orthogonal arrays and analysis of variance (ANOVA) have been used to determine the effects of treatment factors on characteristic properties of the samples (Jeyapaul, Shahabudeen, & Krishnaiah, 2005; Oztop, Sahin, & Sumnu, 2007; Unal & Dean, 1990). Orthogonal tests use reduced number of experiments to optimise processing conditions, thereby reducing the cost and time of experimental trials.

Preliminary results from the single factor test (section 3.4.1) were used to design the experiments in this phase. Based on the single factor test results (section 3.4.1), three fermentation temperatures (40 °C, 43 °C, 45 °C), three protein concentrations (3%, 5%, 7%, w/v) and fermentation times (8, 10, 12 h) were selected as shown in Table 3.3.

experiments to optimise the fermentation of refined pea protein paste-coconut milk beverage				
Loval	Factor A (fermentation	Factor B (fermentation time,	Factor C (protein	
Level	temperature, °C)	h)	concentration, %)	
1	40	8	3	
2	43	10	5	
3	45	12	7	

Table 3.3 Three levels of fermentation conditions with three factors for the orthogonal experiments to optimise the fermentation of refined pea protein paste-coconut milk beverage

The standardised orthogonal experimental design uses an L9 (3^3) array with three columns and nine rows (Zhang, Chen, & Kirby, 2007). The L9 (3^3) array has eight degrees of freedom with the capacity to use up to three control factors, each at three levels. Thus, nine experimental treatments (formulations) were conducted using a combination of levels for each control factor (fermentation temperature, fermentation time and pea protein concentration) as shown in Table 3.4. Levels of the three factors were selected based on the previous single factor experimental results (section 3.4.1). Nine formulations (experiments 1-9) and corresponding samples were developed in this phase. To determine the effects of protein concentration on the fermented beverages, the samples were analysed for VCCs and a semi-trained sensory panel evaluated the beverages at the end of the fermentation period.

Experiment	Factor A (fermentation temperature, °C)	Factor B (fermentation time, h)	Factor C (protein concentration, %)
1	40	8	3
2	40	10	5
3	40	12	7
4	43	8	5
5	43	10	7
6	43	12	3
7	45	8	7
8	45	10	3
9	45	12	5

Table 3.4 Orthogonal tests for refined pea protein paste-coconut milk fermentation

Note: Orthogonal design was generated by SPSS Version 22 (IBM[™], New York, USA) software.

For experiments 1-3 (Table 3.4), 3%, 5% and 7% refined pea protein paste (w /v) in 100 mL coconut milk samples, were fermented (40 °C) in a water bath for 8, 10 and 12 h, respectively. For experiments 4-6, samples containing 5%, 7% and 3% refined pea protein in the coconut milk were fermented at 43 °C for 8, 10 and 12 h. The setup for experiments 7 and 9 was similar to experiments 1-6, samples containing 7%, 3% and 5% refined pea protein in the coconut milk were fermented at 45 °C for 8, 10 and 12 h.

3.4.2.2 Semi-trained sensory panel sensory evaluation

The aim of the semi-trained sensory panel sensory evaluation was to determine the most accepted of the three beverage samples. The semi-trained sensory panel consisted of six participants who consumed or were familiar with plant fermented beverages. Before evaluation, panelists were asked to read the information sheet and signed the participant consent form (Appendix D) which had been approved by Massey University Human Ethics Committee (4000020456). The semi-trained sensory panel evaluated nine fermented pea protein-coconut milk beverages (Table 3.4) for appearance, aroma, sourness, sweetness, mouthfeel, after-taste

and the beany flavour. Additional information was collected using the 9-point hedonic rating scale for the overall acceptability, in the sensory laboratory at Massey University, Auckland Campus. For the sensory evaluation, 20 mL chilled (4 °C) samples were served to the participants in 25 mL plastic cups. Participants were required to rinse their palate with still mineral water between each sample.

3.4.3 Consumer sensory evaluation

The samples selected by the semi-trained sensory panel were subjected to further sensory evaluation by consumer sensory panelists (n=90) using the 9-point hedonic rating scale on three different occasions. The main purpose of the consumer sensory evaluation was to select the most acceptable fermented pea protein coconut beverage by a large number of consumer sensory participants. The consumer panelists evaluated the three fermented beverages selected by the semi-trained sensory panel (section 3.4.2.2). The samples were evaluated for appearance, aroma, sourness, sweetness, flavour, mouthfeel, after-taste and overall acceptability of each sample. Before sensory evaluation, the samples were coded by 3-digit random numbers generated by R Studio software (R-Studio, USA). In this section, consumer sensory panelists were presented with the same ethics documents as described in section 3.4.2.2.

3.4.4 Analysis of the physicochemical characteristics of pea protein-coconut milk beverage during fermentation

Various parameters (pH, T.A., VCCs, colour, sugar, organic acid, crude protein) were determined during the fermentation of the final pea protein-coconut milk beverage (sample 1: the result from section 3.4.1, 3.4.2 and 3.4.3). The methods for measurement of pH and enumeration of VCCs were described in section 3.4.1.1 and 3.4.1.4, respectively. The methods used for the analysis of T.A., sugar, organic acid, crude protein and measurement of colour are described in the subsequent sections.

3.4.4.1 Determination of titratable acidity

Titratable acidity was determined by acid-base titration using 0.1% phenolphthalein (Univar, Ajax Finechem Pty Ltd, NZ) as an indicator (AOAC 947.05, 2005). Standardized 0.1 M sodium hydroxide (NaOH) (Univar, Ajax Finechem Pty Ltd, NZ) was used to titrate against 5 g of

fermented pea protein-coconut milk beverage which had been pre-weighed on an analytical balance (Appliance Check, NZ) and mixed with 20 mL of distilled water. About 2 mL (3-4 drops) of phenolphthalein solution (1%) (Univar, Ajax Finechem Pty Ltd, NZ) was added to the mixture and swirled. The test mixture was titrated against NaOH until the first persistent (30 s) pink colour and the end-point of pH 8.2 was achieved. Calculated titratable acidity was expressed in grams of lactic acid per liter of sample. The analysis of titratable acidity was conducted in duplicate and the experiment was repeated twice.

% Lactic acid = $\frac{\text{volume of NaOH used(ml)} \times 0.0090}{\text{sample weight (g)}} \times 100$ 1 mL 0.1 M NaOH = 0.009 g Lactic acid; 1 mL of sample \approx 1 g of sample

3.4.4.2 Analysis of sugar

Sugar affects the rate of fermentation reactions. Three percent sugar could speed up fermentation (Corona et al., 2016; Magalhaes et al., 2010). Therefore, analysis sugar content is important to study fermentation process. Determination of sucrose, glucose and fructose in fermented pea protein-coconut milk beverage was performed by high-performance liquid chromatography (HPLC) system following the method of Stadie (2013) with some modifications. The HPLC system consisted a model LC-10AT HPLC (Shimadzu Corp, Japan), column oven (CTO-10AS, Shimadzu Corp, Japan), autoinjector (SIL-10A, Shimadzu Corp, Japan) and a system controller (SCL-10A, Shimadzu Corp, Japan) equipped with an Ultra Violet (UV) detector (SPD-10A, Shimadzu Corp, Japan) and a Refractive Index (RI) detector (RID-10A, Shimadzu Corp, Japan). Distilled water which was previously filtered through anylon membrane filter 0.22 um (\emptyset = 47 mm) (Merck, Germany) and degassed with ultrasonic bath (Bandelin Sonorex Super RK510, Germany) was used as the mobile phase at 0.5 mL/min, a separation Rezex RCM- Monosaccharide, RCM Ca^{2+} (8% cross-linked resin) column (300 \times 7.8 mm) was used for the determination at 40 °C. Before analysis, a series of standard sugars: sucrose (≥99.5%, Sigma Aldrich, NZ) standards, glucose (≥99.5%, Sigma Aldrich, NZ) standards and fructose (≥99%, Sigma Aldrich, NZ) were prepared as external standards. The concentration of sucrose and fructose standards in distilled water were 0.5%, 1%, 1.5%, 2%, 2.5%, 3% (w/v in water) and the concentration of glucose standards were 0.2%, 0.4%, 0.6%, 0.8%, 1%, 1.2% (w/v). All standards and test samples were previously filtered through 0.22 μ m syringe filters (Merck, Germany) and stored in 2 mL vials (Shimadzu Corp, Japan). Automatic uploading (1 μ l) of samples was conducted in duplicate and each sugar was identified and quantified by comparing with retention times and peak areas of the standards using Shimadzu LC solutions software (Shimadzu Prominence, Japan). The concentration of sugars in the beverage was interpolated from the standard calibration curves.

3.4.4.3 Analysis of organic acids

Lactic acid and acetic acid in the fermented pea protein-coconut milk beverage were analysed by HPLC using UV detection, according to Stadie (2013) with minor modifications. The HPLC model LC-10AT (Shimadzu Corp, Japan) equipped with an autoinjector (SIL-10A, Shimadzu Corp, Japan), column oven (CTO-10AS, Shimadzu Corp, Japan), system controller (SCL-10A, Shimadzu Corp, Japan) and a dual detection system consisting of a Refractive Index (RI) detector (RID-10A, Shimadzu Corp, Japan) and an Ultra Violet (UV) detector (SPD-10A, Shimadzu Corp, Japan) was used for the analyses. A separation Rezex ROA-Organic Acid (8% cross-linked resin) column (300×7.8 mm) was used for the analyses at 40 °C, sulphuric acid (Fisher Scientific, UK) (0.005 N) was used as the mobile phase at a flow rate of 0.5 mL /min. Prior to analysis, standard solutions and test samples were filtered through 0.20 µm syringe filters (Terumo, Australia) and kept stored in 2 mL vials (Shimadzu Corp, Japan) for chromatographic analysis. Automatic injections (10 µl) were performed in duplicate. Individual organic acids were identified and quantified by comparison of their retention times and peak areas respective to their standards (lactic acid, 252476 ≥95%, Sigma Aldrich, NZ; acetic acid, 2789, ≥99.5%, Fisher scientific, UK). The concentrations of lactic acid and acetic acid standard used were 0.3125%, 0.625%, 1.25%, 2.5%, 5% (w/v). Peak areas were integrated using Shimadzu LC Solutions Software (Shimadzu Prominence, Japan). The quantification of organic acids was performed using calibration curves obtained from the standard compounds.

3.4.4 Analysis of crude protein

The quantity of protein in the pea protein coconut fermented beverage was analysed using the Kjeldahl method (Lynch & Barbano, 1999). The first step was the digestion of the sample. Samples of the fermented pea protein-coconut milk beverage were separately weighed (0.5-1 g) and transferred into respective digestion tubes with two Kjeltabs tablets (Univar, Ajax Finechem Pty Ltd, NZ). A blank digestion (control) was also included in the analysis of crude protein. The samples were digested at low temperature, gradually increasing the intensity of

heat to 420 °C using the Kjeltec[™] 8400 digestion unit (FOSS, Denmark). Digestion was stopped when the samples were clear which took about 4 h. After digestion, the samples were removed carefully from the heating unit and allowed to cool distilled water (70 mL) was then added to each tube and mixed.

The next step was the distillation of the samples using Kjeltec 8400 (FOSS, Denmark) which was done according to the instructions of the manufacturer. When the distillation was completed, the distilled samples were titrated against 0.1 M HCl to a grey-mauve end-point (reference).

Calculation used to determine % nitrogen and protein content in samples:

% Nitrogen = $\frac{(A \times B) \times 14 \times 100}{1000 \times C}$

Where A = mL HCl used; B = exact molarity HCl; C = weight (g) of test sample used;

Protein (%) = % Nitrogen x conversion factor

Note: the conversion factor is 6.25.

3.5 Phase 3: Stability of the fermented pea protein-coconut milk beverage

Four 500 mL pea protein paste coconut milk mixtures containing 3% refined pea protein were prepared and fermented at 40 °C /8 h using the lactic starter culture 20 DCU VEGE 053 LYO as previously described in section 3.4.1.1. After fermentation, the four bottles of fermented pea protein-coconut milk beverage products were stored at 4 °C (Fisher & Paykel, NZ) for 21 days. One sample (500 mL) was retrieved from chilled storage for determination of various parameters (pH, colour, titratable acidity, VCCs, sugar, organic acid, crude protein) after storage for 1, 7, 14 and 21 days. The other samples were also evaluated by a semi-trained sensory panel (n=15) at day 1, 7, 14 and 21 as described in section 3.4.2.2.

3.6 Statistical analysis of data

The data were analysed by the SPSS Version 22 (IBMTM, New York, USA) software using the univariate of General Linear Model (GLM) analysis of variance (ANOVA). Data were expressed as mean \pm standard deviation (SD) or \pm standard error of the mean (SEM) and were tested for normality and homogeneity using the Kolmogorov-Smirnov and Levene Test at 95% confidence level. Non-normally distributed data were log transformed to obtain normality. Normally distributed data were further analysed by one-way analysis of variance (ANOVA). ANOVA was used to investigate the effects of the concentration of pea protein, fermentation temperature and fermentation time on the physicochemical, microbiological and sensory properties of the product at α =0.05. The least significant difference (LSD) multiple comparisons test was applied to separate significant differences between group means at 95% confidence interval.

4. RESULTS AND DISCUSSION

4.1 Phase 1: Refined commercial pea protein powder

4.1.1 Colour of refined commercial pea protein powder

The appearance of food is generally the first impression the consumer encounters. In addition, the appearance of food can be an indicator of freshness, preservation state and flavour expectation (Hutchings & Hutchings, 1999; Chung et al., 2016). The purification step aimed to transform the yellow coloured commercial pea powder to a white coloured refined pea protein paste. Then to broaden its applications in food, including fermented dairy-like beverages. For dairy-like beverages, whiteness is one of the most important factors for consumer acceptance (Kwok et al., 1999). It was therefore important to measure the colour of the purified pea protein and compare its colour to the commercial pea powder (Figure 4.1). Figure 4.1 shows that the colour of the commercial pea powder was transformed into a white refined pea paste.



Figure 4.1 Images of commercial pea protein powder (a) and refined pea protein paste (b) Note: Images were captured by iPhone XR, Apple Inc., California, USA

Figure 4.2 compares the colour of the commercial pea protein powder with the purified pea protein paste. The lightness (L*) increased from 76.88 ± 0.11 to 81.14 ± 0.76 , redness (a*) decreased from 3.08 ± 0.02 to 0.35 ± 0.06 and yellowness (b*) decreased from 16.32 ± 0.09 to 6.86 ± 0.12 (p<0.05) (Appendix E). The higher L* values of the refined pea protein paste (pH 5) reflected a lighter and white colour than the commercial pea protein powder. The positive a* and b* values of the purified paste indicated that the paste was a lighter yellowish-red colour than the commercial pea powder. The colour change may be attributed to the salt extraction, with the yellow pigment from the commercial pea protein powder being reduced or eluted during purification (Taylor, Fields, & Elder, 2004).



Figure 4.2 Mean colour values of commercial pea protein powder and refined pea protein paste

Notes: Error bars are means \pm standard deviation; n=3. L* represents lightness (0 is black and 100 is diffuse white); a* is the greenness /redness (negative values represent green, positive values represent red) and b* is the blue /yellow (negative values indicate blue, positive values are yellow) (Alqahtani, Aljurais, & Alshaafi, 2012).

Sumner, Nielsen and Youngs (1981) reported a higher colour values of the pea paste (pH 4.5) than the present study. On the contrary, the refined pea protein (pH<4) in the study of Kizer, Renninger and Stiles (2019) had lower colour values compared to the present study. The differences in the colour values in these different studies were probably attributed to different acidic levels of the refined pea proteins. Because Khan and Farooqui (2011) stated that anthocyanins produce a red to yellow colour when the environment changes from alkaline to acidic. The yellowish-red colour was expected in the products due to the acidic property of purified pea protein (Stintzing & Carle, 2004).

4.1.2 Protein content of purified pea protein

Figure 4.3 shows the protein concentration of the commercial pea protein powder and the purified pea protein paste. The protein concentration of refined pea protein paste was $17.31\pm0.32\%$, which was lower than the protein concentration of the commercial pea powder (77.44±0.56%) (p<0.05). The differences in the protein levels of the two products were probably attributed to their different water content. Comparing with the dry commercial pea

powder which has low water content, the refined pea protein paste has a large amount of water in it. As predicted, the protein concentration of dry refined pea protein paste ($92.63\pm1.20\%$) was higher than the commercial pea powder ($77.44\pm0.56\%$) by around 15% after the extraction process due to the protein enrichment (p<0.05) (Appendix E).



Figure 4.3 Mean pea protein concentrations of commercial pea protein powder, refined pea protein paste and dry refined pea protein paste

The protein concentration of the refined pea protein paste was similar to the patent of Kizer, Renninger and Stiles (2019). In their patent, the mean pea protein concentration of the refined pea protein paste was 17% (w/w). The results were similar to those of our study because we used same raw material (Roquette S85F) and the same methods for refining and extraction.

However, Boye et al. (2010) reported lower protein concentrations (63.9-81.7%) of pea protein paste using isoelectric precipitation was, which were lower than our results (92.63 \pm 1.20%). The differences in the protein concentrations may be attributed to the different methods used in their studies. Boye et al. (2010) only used the isoelectric precipitation method to refine pea protein which was suitable for crude extraction of protein. Whereas, we used both isoelectric precipitation and salt extraction methods to refined the pea protein (Makri, Papalamprou, & Doxastakis, 2005). The salt extraction method combined with the isoelectric precipitation method could assist in producing higher concentration refined protein (Murray, Pizzorno, &

Notes: Error bars are means \pm standard deviation; n=3.

Pizzorno, 2005). The protein concentration obtained in this study (92.63±1.20%) was slightly higher than the study of Sumner, Nielsen and Youngs (1981). In their study, the protein content of pea protein isolate ranged from 83 to 90% for the pea protein isolates. The addition of salt extraction method and the higher protein concentration of the original sample in the present study may contribute to the differences in protein content of the study by us and by Sumner, Nielsen and Youngs (1981). The high protein concentration of dry refined pea protein paste suggested that the purification method was effective in purifying the commercial pea powder and improving the protein content.

4.1.3 Summary

The purification process produced a white (L*: 81.14 ± 0.76) refined pea protein paste. The protein concentrations of wet refined pea paste and dry refined pea paste were $17.31\pm0.32\%$ and $92.63\pm1.20\%$, respectively. The refined pea protein paste was added to coconut milk to develop a new dairy-like fermented beverage.

4.2 Phase 2: Development of a fermented pea protein-coconut milk beverage.

In Phase 2, the refined pea protein paste was added to organic coconut milk for fermentation with a starter culture VEGE 053 LYO. Single-factor tests and orthogonal experiments were conducted to select (optimise) the fermentation conditions of the fermented pea protein-coconut milk beverage. Semi-trained sensory panel sensory evaluation and consumer sensory evaluation were conducted to select a fermented pea protein-coconut milk beverage with the most acceptable sensory properties. Various parameters (pH, T.A., VCCs, colour, sugar, organic acids and protein content) were determined to characteristics the fermented milk.

4.2.1 Selection (Optimisation) of the fermentation conditions using the single factor test

Fermentation conditions comprising the starter culture used, type of raw materials, sugar concentration, fermentation temperature and time have important effects on the fermentation process (Wang et al., 2015). Therefore, to develop a full-bodied and stable fermented beverage, the fermentation conditions must be optimised. The starter VEGE 053 LYO 200 DCU culture used was specific for vegetal and plant fermentation (Dupont company, Auckland, New Zealand). Therefore, in this experiment on the development of fermented pea protein-coconut milk beverage, the fermentation temperature, fermentation time and pea protein concentration were the main factors studied for optimisation.

4.2.1.1 Effect of temperature on fermentation

The effect of five temperatures (35 °C, 37 °C, 40 °C, 43 °C and 45 °C) on the fermentation of the pea protein-coconut milk were investigated. The pea protein-coconut milk fermentation process was monitored by measuring pH (Yoon, Woodams, & Hang, 2005). Figure 4.4 shows that fermentation at higher temperatures accelerated the fermentation process. After fermentation for 12 h, the pH decreased from 6.2 ± 0.00 to 4.19 ± 0.07 , 4.13 ± 0.05 , 4.09 ± 0.06 , 3.98 ± 0.08 , 3.92 ± 0.05 respectively, in samples fermented at 35 °C, 37 °C, 40 °C, 43 °C and 45 °C (p<0.05) (Appendix E). The pH decreased slowly during the first 2 h, then decreased rapidly between 2 h and 8 h. After fermentation for 8 h, the pH gradually decreased up to 12 h.



Figure 4.4 Mean pH of refined pea protein paste-coconut milk during fermentation at different temperatures for 12 h

Notes: Error bars are means \pm standard deviation; n=3.

The decrease in pH during fermentation was caused by the production of organic acids, during the fermentation process, with glucose being converted to lactic acid, CO₂ and ethanol (Makarova et al., 2006; Moraes Filho et al., 2016; Østlie et al., 2003). The optimum growth temperature of LAB varies between species and strains of cultures. In soymilk fermented by *Lactobacillus plantarum* BG 112, the optimum temperature was 37 °C, while *Lactobacillus acidophilus* LA 3 showed better growth at 31 °C (Moraes Filho et al., 2016). In contrast, *L. reuteri* SD 2112 exhibited rapid growth in milk when incubated at 37 °C and 45 °C (Østlie et al., 2003). However, in the present study involving fermentation of coconut milk with the VEGE 053 LYO starter culture, rapid decreases in pH were observed at higher (40 °C, 43 °C and 45 °C) temperatures. From the results, it may be concluded that higher temperature was supported better growth of the VEGE 053 LYO in the coconut milk.

After fermentation 12 h, the most significant pH decrease $(6.2\pm0.00 \text{ to } 3.92\pm0.05)$ was recorded in the sample fermented at 45 °C. Therefore, 45 °C was selected for the fermentation of pea protein-coconut milk beverage in the next two experiments (section 4.2.1.2 and 4.2.1.3).

4.2.1.2 Effect of refined pea protein paste concentration on changes in pH of the fermentation mixtures

Five concentrations of refined pea protein paste (3%, 5%, 7%, 9% and 11%) were used to evaluate the effect of the pea protein concentration on the rate of pea protein-coconut milk fermentation at 45 °C. The rate of fermentation was determined by measuring changes in the pH of the fermentation mixtures.

The results showed that there were no significant differences of the pea protein paste concentrations (3%, 5%, 7%, 9% and 11%) on the changes in pH of the mixtures during fermentation (p>0.05) (Appendix E). After fermentation, the average pH of the beverages was 4.04 ± 0.08 , 4.09 ± 0.06 , 4.13 ± 0.06 , 4.19 ± 0.05 and 4.26 ± 0.07 for the respective treatments. The fastest change in pH was recorded in the mixture that contained the lowest pea protein paste concentration in the fermented pea protein-coconut milk beverages (Figure 4.5).



Figure 4.5 Mean pH of refined pea protein paste-coconut milk during fermentation by contained various pea protein concentrations

Notes: Error bars are means \pm standard deviation; n=3.

The effect of the pea protein concentration on the decrease in pH can be mainly attributed to the metabolism of the sugar into organic acids in the fermentation mixture. The sugar content in pea protein is low, around 1-2% (Sánchez et al., 1998). Thus, the different pea protein

concentrations are likely to have a limited effect on pH change of the fermentation. In the present study, the fermentation was mostly induced by the sugar content of coconut milk.

Several studies have investigated the effects of different protein concentrations on the fermentations. Angelov et al. (2006) fermented a whole-grain oat substrate with lactic acid bacteria to produce a drink. Similar to our study, they found that lower concentrations (4.0–5.5%) of oat flour in the mash were more appropriate for intensive fermentation. Also, Denkova et al. (2015) used fermentation to produce a pea protein-cow milk yogurt. They found that the lower concentrations (2-4%) of pea protein in the yogurt had a faster rate of fermentation. In contrast, Mauro and Garcia (2019) reported higher concentration (1:3 w /v) of coconut pulp in water better for the growth of *Lactobacillus reuteri* LR 92 grown in coconut milk beverage.

In this experiment, the highest pH change $(6.20\pm0.00 \text{ to } 4.04\pm0.08)$ was obtained in the mixture containing 3% pea protein. Therefore, 3% pea protein concentration was selected for the subsequent experiments (4.2.1.3).

4.2.1.3 Effect of fermentation time on viable cell counts

Fermentation time is one of the key factors affecting the overall characteristics of fermented beverages. It is therefore important to determine the optimum fermentation times to develop full-bodied beverages (Montel, Masson, & Talon, 1998). The VCCs in the 3% pea protein paste-coconut milk mixture were determined during fermentation for 24 h at 45 °C (Figure 4.6). The cell counts increased rapidly during the first 8 h, from 5.48 ± 0.15 log CFU /mL to 8.63 ± 0.25 log CFU /mL (p<0.05), and then remained stable up to 12 h. From 12 h to 24 h, the cell counts decreased rapidly to about 7 log CFU /mL (p<0.05) (Appendix E).

Previous studies reported similar growth trends of the LAB in plant base fermented beverages including soymilk (Tsangalis et al., 2002; Telang et al., 2010). During fermentation of soymilk at 37 °C for 24 h, the growth of *Lactobacillus acidophilus* increased from 4.67×10^5 CFU /mL to 1.99×10^9 CFU /mL in the first 12 h and then decreased. Tsangalis et al. (2002) reported rapid exponential growth of *bifidobacteria* in soymilk fermentation in the first 12 h at 37 °C, while, Chun et al. (2008) obtained the exponential growth phase in the first 6 h of soymilk fermentation at 37 °C using single or mixed cultures of *S. infantarius and Weissella* sp 4.



Figure 4.6 log CFU /mL of starter culture during fermentation of refined pea protein paste-coconut milk for 24 h at 45 $^{\circ}\mathrm{C}$

Notes: Error bars are means \pm standard deviation; n=3.

In the present study, the highest VCCs were obtained at 8 h of fermentation and these remained stable up to 12 h (Pitt & Hocking, 2009). Thus, the three fermentation times (8, 10 and 12 h) which recorded the highest VCCs were used in the orthogonal test.

4.2.2 Selection (Optimisation) of the fermentation conditions

4.2.2.1 Orthogonal test for bacterial growth

The fastest growth of starter culture was one of the criteria to design optimum fermentation conditions. Based on the previous single factor test results (Section 4.2.1), the experimental design in the first orthogonal test was a 3 x 3 x 3 factorial design with three fermentation temperatures (40 °C, 43 °C and 45 °C), three pea protein concentrations (3%, 5% and 7%) and three fermentation times (8, 10 and 12 h). There were 9 experimental samples under the 9 fermentation condition combinations. The response variable was the VCCs of the starter culture VEGE 053 LYO in each sample.

An L9 (3³) orthogonal table and test results are shown in Table 4.1. Different fermentation combinations (experiments 1-9) produced different VCCs. The quantity of bacteria in the nine fermented pea protein-coconut milk beverages ranged from 8.00±0.28 log CFU /mL to

 $8.78\pm0.21 \log \text{CFU}/\text{mL}$. Sample 1, which contained 3% pea protein and fermented at 40 °C /8 h had the highest VCCs ($8.78\pm0.21 \log \text{CFU}/\text{mL}$). While the cell counts of sample 9 were the lowest ($8.00\pm0.28 \log \text{CFU}/\text{mL}$). Table 4.1 indicates that the optimum fermentation condition of the fermented pea protein-coconut milk beverage was A1B1C1 (k1> k2 and k3). It can be explained that 3% refined pea protein in the pea protein-coconut milk mixture fermented at 40 °C /8 h was the best fermentation condition for the bacteria growth (Table 4.1). The size of the ranges was $S_A>S_B>S_C$ (0.28>0.27>0.20). It revealed that fermentation temperature had the highest impact on the bacterial quantity of the culture. Meanwhile, pea protein concentration had the smallest effect on the VCCs of fermented pea protein-coconut milk beverage.

Experiments	Factor A (fermentation temperature)	Factor B (fermentation time)	Factor C (protein concentration)	Mean cell counts /log CFU /mL
1	40 °C	8 h	3 %	8.78±0.21
2	40 °C	10 h	5 %	8.54±0.32
3	40 °C	12 h	7 %	8.43±0.44
4	43 °C	8 h	5 %	8.65±0.33
5	43 °C	10 h	7 %	8.18±0.22
6	43 °C	12 h	3 %	8.52±0.15
7	45 °C	8 h	7 %	8.51±0.36
8	45 °C	10 h	3 %	8.41±0.20
9	45 °C	12 h	5 %	8.00±0.28
\mathbf{K}_1	25.75	25.94	25.71	
K_2	25.35	25.14	25.20	
K_3	24.92	25.11	25.11	
\mathbf{k}_1	8.58	8.65	8.57	
k_2	8.45	8.38	8.40	
k3	8.31	8.37	8.37	
S	0.28	0.27	0.20	

Table 4.1 Effects of temperature, time and protein concentration on the fermentation of refined pea protein paste-coconut milk using orthogonal test

Notes: Orthogonal design generated by SPSS Version 22 (IBM[™], New York, USA) software.

K is the sum of mean cell counts (log CFU /mL) of the same level factors; k is the K divided by 3; S is the size of the range of k (Li, 2014; Deesuth et al., 2012; Khongsay et al., 2012).

According to Pereira, Maciel and Rodruiguez (2011), the growth of microorganisms depends on various factors, including the pH of the substrate, temperature and type of starter culture. As the present study, many previous studies (Li, 2014; Deesuth et al., 2012; Khongsay et al., 2012) have applied orthogonal design and data analysis methods to optimise fermentation conditions. Orthogonal tests have been used in various studies to optimise fermentation conditions. Li (2010) used single-factor test and orthogonal test to optimise the culture conditions of *Saccharomy cescerevisiae*. Deesuth et al. (2012) and Khongsay et al. (2012) optimised the parameters for bioethanol production from sweet sorghum juice by *Saccharomyces cerevisiae* NP using an orthogonal array design. Statistical methods can provide a comprehensive and objective analysis of results (Mauro & Garcia, 2019; Meena et al., 2014).

4.2.2.2 Sensory evaluation

The acceptability of food is an important reference indicator by consumers which affects new product development (Jarvis, MacKenzie & Podsakoff, 2003). Sensory evaluation was conducted by a semi-trained sensory panel (n=18) to evaluate and screen the 9 experimental samples (section 4.2.2.1) for the appearance, aroma, sourness, sweetness, mouthfeel, after-taste, beany flavour and overall acceptability. The semi-trained panellists used the 9-point hedonic rating scale on likability (Appendix E).



Figure 4.7 Mean sensory acceptability scores for the pea protein-coconut milk fermented milk

Notes: Hedonic scaling: 1-9 with 1 as lowest and 9 the highest; Error bars are means \pm standard deviation, n=3.

Figure 4.7 shows the results of the sensory evaluation of 9 samples of fermented pea proteincoconut milk beverages. The overall acceptability scores for all the 9 samples ranged from 4.00 ± 0.00 to 6.50 ± 0.50 (Appendix E). Samples 1, 6 and 7 obtained higher scores (p<0.05) than the other six samples (Figure 4.7). Sample 5 received the lowest mean acceptability sensory score (4.00 \pm 0.00), which contained 7% pea protein and was fermented at 43 °C /10 h. The characteristic beany flavour of the pea protein was not detected by the semi-trained sensory panel in all the samples. This suggested that fermentation was able to reduce or remove the beany flavour. However, the volatile compounds that cause the beany flavour in pea protein and related products were not analysed in this study. It is therefore desirable to analyse the compounds in future studies.

Results in Table 4.2 show that the optimum fermentation condition for fermented pea proteincoconut milk beverage was the fermented sample contained 3% pea protein in the fermentation mixture fermented at 40 °C /8 h and it received the highest overall sensory acceptability score (6.50 ± 0.50).

Experiments	Factor A (fermentation temperature)	Factor B (fermentation time)	Factor C (protein concentration)	Mean semi-trained sensory panel sensory score
1	40 °C	8 h	3 %	6.50±0.50
2	40 °C	10 h	5 %	5.50 ± 0.50
3	40 °C	12 h	7 %	4.67±0.15
4	43 °C	8 h	5 %	5.67±0.15
5	43 °C	10 h	7 %	4.00±0.00
6	43 °C	12 h	3 %	6.20±0.50
7	45 °C	8 h	7 %	6.17±0.35
8	45 °C	10 h	3 %	5.83±0.35
9	45 °C	12 h	5 %	4.50±0.20
K_1	16.88	18.34	18.53	
K_2	15.87	15.33	15.67	
K ₃	16.50	15.05	15.05	
\mathbf{k}_1	5.63	6.11	6.18	
k_2	5.29	5.11	5.22	
k_3	5.50	5.02	5.02	
S	0.34	1.10	1.16	

 Table 4.2 Effect of fermentation conditions on overall acceptability of fermented pea protein
 -coconut milk beverage

Notes: Orthogonal design generated by SPSS Version 22 (IBM[™], New York, USA) software. K is the sum of mean cell counts (log CFU /mL) of the same level factors; k is the K divided by 3; S is the size of the range of k (Li, 2014; Wang et al., 2010; Deesuth et al., 2012; Khongsay et al., 2012).

The size of the range was $S_C > S_B > S_A$ (1.16>1.10>0.34), which suggested that the pea protein concentration had the highest impact on the acceptability of the fermented pea protein-coconut

milk beverage. Whereas fermentation temperature has the lowest impact on the acceptability of the fermented pea protein-coconut milk beverage (Table 4.2).

4.2.3 Consumer sensory evaluation of fermented pea protein-coconut milk beverage during fermentation at 40 °C /8 h.

The purpose of the consumer sensory evaluation was to determine the acceptability of the three samples by a larger sensory group (n=90). Three samples (1, 6, 7; Table 4.2) which received the highest sensory scores for acceptability from the semi-trained sensory panel sensory evaluation were presented to consumer sensory panellists for evaluation in this section.



Figure 4.8 Mean sensory scores of fermented pea protein-coconut milk beverage

Notes: Sample 1 = contained 3% pea protein and was fermented at 40 °C /8 h; Sample 6 = contained 3% pea protein and was fermented at 43 °C /12 h; Sample 7 = contained 5% pea protein and was fermented at 45 °C /8 h; Hedonic scale: 1-9 with 1 as lowest and 9 highest; Error bars = \pm SD; n=90.

The results of consumer sensory evaluation for appearance, aroma, sourness, sweetness, flavour, mouthfeel, after-taste and overall acceptability of the three fermented beverages are shown in Figure 4.8. The spider plot in Figure 4.8 shows that the different fermentation conditions had minimal effects on the sensory characteristics of the fermented beverage. Except for sweetness, the score of other characteristics of sample 1 were all higher than the accordance characteristics score of samples 6 and 7 (p<0.05) (Appendix E). Sample 1 received a higher

overall sensory score (6.2) than sample 6 and sample 7 which have the same overall sensory score (5.5). Therefore, it can be concluded that sample 1, which contained 3% pea protein and fermented at 40 $^{\circ}$ C /8 h was best accepted by the consumers. Sample 1 was therefore selected as the final fermented pea protein-coconut milk beverage (Figure 4.9). The optimum fermentation conditions (3% pea protein, 40 $^{\circ}$ C, 8 h) were used in the following section 4.2.4.

The bitter taste and beany flavour of the pea protein were not detected by the sensory panellist after the refined pea protein fermented with coconut milk. That could be attributed to the fermentation removed the beany /bitter flavour of pea protein.



Figure 4.9 Appearance of fresh final fermented pea protein-coconut milk beverage Note: Image was captured by iPhone XR, Apple Inc., California, USA.

Several previous studies have analysed the sensory characteristics of plant-based fermented beverages. For example, Menezes et al. (2018) studied the fermentation of *Lactobacillus paracasei* LBC-81 in a maize-based substrate by a LAB-yeast co-culture. The overall sensory acceptability of their products ranged from 5.07 to 5.45, which were lower than the present study. In contrast, higher sensory acceptability scores (ranged from 7.28 to 7.8) were shown for a kefir grain fermented cocoa beverages (Puerari, Magalhães, & Schwan 2012). The differences in the scores of overall sensory acceptability might be attributed to the fermentation of Menezes et al. (2018) did not add flavours and sweetness.

Additionally, the general improvement of the sensory profile of fermented products by added culture has been widely reported (Kaczmarska et al., 2018; Meinlschmidt et al., 2016; Schindler
et al., 2011; Yaakob et al., 2012). According to Obinna-Echem, Kuri and Beal (2014) and Edema and Sanni (2008), the use of starter cultures improved the aroma of fermented maize due to the release of aromatic compounds. They also indicated the added starter culture produced a better flavour and aroma profile.

4.2.4 Characteristics of pea protein-coconut milk fermentation during fermented at 40 $^{\circ}\mathrm{C}$ /8 h

4.2.4.1 Acidity

The acidity (pH and T.A.) of fermented pea protein-coconut milk beverage was determined during fermentation for at 40 °C /8 h (Figures 4.10). The pH of fermented pea protein-coconut milk beverage decreased from 6.15 ± 0.13 to 4.29 ± 0.02 , while T.A. increased (p<0.01) steadily from $0.09\%\pm0.01$ to $0.52\%\pm0.03$ during fermentation (p<0.05) (Appendix E).



Figure 4.10 Mean pH and titratable acid of fermented pea protein-coconut milk beverage during fermentation at 40 $^{\circ}C$ /8 h

Notes: T.A. = Titratable Acidity; Error bars are means \pm standard deviation; n=3.

Our results are similar to previous studies by Garcia et al. (2006) and Sabokbar and Khodaiyan (2015). Fermentation of plant-based beverages produces organic acids such as lactic acid and acetic acid (Stadie et al., 2013; Laureys & De Vuyst, 2014). The concomitant increase in T.A. and decrease in pH may be attributed to the metabolism of sugars in fermented pea protein-

coconut milk beverages to organic acids by LAB via either the homofermentative and heterofermentative pathways or the fructose-6-phosphate phosphoketolase pathway (Hamad, 2011; Mauro & Garcia, 2019).

The pattern of changes in pH and titratable acidity (T.A.) differ according to the type of microorganism used and different fermentation conditions. The amount of acid produced in fermented beverages is dependent on the growth of bacteria or the type of starter culture used (Kazakos et al., 2016; Puerari et al., 2012). Mauro and Garcia (2019) studied the fermentation of coconut milk fermented by *L. reuteri* LR 92 or DSM 17938. Coconut milk was fermented with *L. reuteri* DSM 17938 to pH 3.32, while that fermented by *L. reuteri* LR 92 had pH 4.28 after 48 h at 37 °C. Donkor et al. (2007) reported a progressive decrease in pH and increased acidity during 48 h fermentation of soy yogurt, which was similar to the results observed in the present study. Zare et al. (2012) suggested that the development of acidity might be influenced by the carbohydrate content of the components added. The carbohydrate (concentration 6.34%) in coconut milk may assist the development of acidity during the fermentation.

4.2.4.2 Viable cell counts

The growth of the starter bacteria VEGE 053 LYO during 8 h fermentation is shown in Figure 4.11. During fermentation, the bacteria exhibited steady growth, with the viable cell count increasing from 5.54 ± 0.10 to $8.61\pm0.35 \log$ CFU /mL (p<0.05) (Appendix E).



Figure 4.11 Mean VCCs (log CFU /mL) of fermented pea protein-coconut milk beverage during fermentation at 40 °C /8 h

Notes: Error bars are means \pm standard deviation; n=3.

Telang et al. (2010) and Stadie et al. (2013) reported similar results on VCCs to our study. To reduce the beany flavor of soymilk and obtain a nutritious food product, Telang et al. (2010) used LAB starter culture to ferment the soymilk. Viable cell counts of 10^9 CFU /mL sample were obtained in the soymilk after 12 h of fermentation at 40 °C.

4.2.4.3 Colour

Color is an important attribute of the dairy alternative beverages (Chaturvedula & Prakash, 2011; Pathare et al., 2013). Therefore, it was important to measure the color of the fermented pea protein-coconut milk beverage.





Figure 4.12 Mean L*, a*, b* values of fermented pea protein-coconut milk beverage during fermentation at 40 °C /8 h

As shown in Figure 4.12, the Hunter values (L*, a*, b*) of the fermented pea protein-coconut milk beverage transformed during fermentation (p<0.05). The L* and b* values increased during fermentation from 73.40 ± 0.27 to 74.48 ± 0.26 and 2.98 ± 0.04 to 4.96 ± 0.31 , respectively, while a* value decreased from 0.03 ± 0.01 to -0.02 ± 0.01 . The increasing L* value indicated that the color of the product became lighter, which was probably due to the suppression of ionization or destruction of the pigmented structure of the origins during the fermentation (Haslam, 2003).

Schaffner and Beuchat (1986) used *Streptococcus thermophilus* and *Lactobacillus bulgaricus* to ferment seed extract powder of soy flour, cowpea and peanut. The results showed that fermentation improved the color of the legume powders. But fermentation did not significantly impact the b* values for any of the legume products in their study.

4.2.4.4 Sugars and organic acids in fermented pea protein-coconut milk beverage

The levels of sugars and organic acids in the fermented pea protein-coconut milk beverage during fermentation are shown in Figure 4.13. Sucrose decreased from $2.20\pm0.06\%$ to $1.83\pm0.04\%$ during fermentation (p<0.05) (Appendix E). This result was similar to the study by Menezes et al. (2018) showed that sucrose was consumed during the fermentation of maize-based beverages. Glucose was not detected in the fermented pea protein-coconut milk beverage,

Notes: Error bars are means \pm standard deviation; n=3. L* represents lightness (0 is black and 100 is diffuse white); a* is the greenness /redness (negative values represent green, positive values represent red) and b* is the blue /yellow (negative values indicate blue, positive values are yellow) (Alqahtani, Aljurais, & Alshaafi, 2012).

possibly because it was metabolism to lactic acid by the LAB culture. The concentration of fructose increased from $0.14\pm0.01\%$ to $0.19\pm0.01\%$ after pea protein-coconut beverage fermentation (p<0.05). Fructose increased during fermentation, suggesting that the monosaccharide was produced from the degradation of sucrose by lactic acid bacteria (Laurey & de Vuyst, 2014; Leroi & Pidoux, 1993). The increase in fructose was also reported by Stadie et al. (2013) during water kefir fermentation.

Lactic and acetic acid concentrations increased during 8 h fermentation (p<0.05) (Figure 4.13), which peaked at $1.23\pm0.09\%$ and $2.79\pm0.953\%$ respectively, by the end of fermentation. However, in the study of Menezes et al. (2018), the concentration of acetic acid ranged from 0.1 to 0.2 g/L, which was lower than the present study. The difference in the concentrations of acetic acid between two studies may be related to the variations of microflora composition in start culture and substrate (Hsieh et al., 2012). Production of lactic acid and acetic acid in dairy-free fermentations indicate the existence of homo-fermentative and hetero-fermentative pathways by LAB (Puerari et al., 2012; Stadie et al., 2013).



Figure 4.13 Concentrations of sugars and organic acids during the fermentation of fermented pea protein-coconut milk beverage at 40 °C /8 h

Notes: Error bars are means \pm standard deviation; n=3.

4.2.4.5 Protein content

There was no significant difference in the protein content of fermented pea protein-coconut milk beverage during fermentation (p>0.05). Khetarpaul and Chauhan (1989) reported similar results of crude protein in pearl millet flour during fermentation at 30 °C /72 h. The increased protein catabolism by fermenting microorganisms may account for the loss of protein through ammonia (a byproduct of metabolic deamination) (Assohoun et al., 2013). Our results are contrary to Magalhães et al. (2011) who reported an increase in protein content in Brazilian kefir. Shekib (1994) also reported an increase in the crude protein of fermented lentils and chickpea during fermentation.

4.2.5 Summary

There is an information gap in the optimisation of fermentation techniques for the growth and development of probiotic bacteria in plant-based products (Gupta & Abu-Ghannam, 2012). Results from phase 2 suggested that higher fermentation temperatures ($40 \circ C$, $43 \circ C$ and $45 \circ C$), lower pea protein concentrations (3%, 5% and 7%), and three fermentation times (8, 10 and 12 h) improved the pea protein-coconut milk fermented by VEGE 053 LYO starter culture. Further, fermentation of pea protein-coconut milk containing 3% pea protein concentration at $40 \circ C / 8$ h was the most promising formulation for the fermented beverage (Sample 1). This beverage sample contained the highest quantity of VCCs ($8.78 \log CFU / mL$) and received the highest overall acceptability score of 6.2.

The pH, titratable acidity, VCCs, and colour changed during the fermentation of refined pea protein paste-coconut milk beverage (p<0.05). The protein content of the beverage was stable during fermentation.

4.3 Phase 3: Stability of the fermented pea protein-coconut milk beverage during storage at 4 °C /21 days.

4.3.1 The pH and titratable acid changes of fermented pea protein-coconut milk beverage during storage (4 °C) for 21 days

Results of the pH and titratable acidity (T.A.%) of the fermented pea protein-coconut milk beverage stored at 4 °C for 21 days are presented in Figure 4.14. As expected, the pH of the fermented pea protein-coconut milk beverage decreased from day 1 to day 14 (p<0.05). However, the pH was stable from day 14 to day 21 (p>0.05). Titratable acidity increased (p<0.05) from day 1 to day 14 and remained stable during the last week of storage (p>0.05) (Appendix E).



Figure 4.14 Mean pH and titratable acid of fermented pea protein-coconut milk beverage during the storage (4 °C) /21 days

Notes: T.A. = Titratable Acidity; Error bars are means \pm standard deviation; n=3.

The acidity levels reported here are similar to the study by Akin and Ozcan (2017) who reported a decrease in pH (pH 4.33 to 3.95) during storage for 21 days /4 °C of a fermented pea protein-coconut milk beverage. Du (2018), also reported decreases in pH of berry-containing kefir during 28 days /4 °C. The reduction in pH suggested the continued metabolism of sugars to organic acids by the starter culture during storage (Yilmaz et al., 2006; Leite et al., 2013).

However, during the last seven days (day 14 to day 21), both pH and T.A. were stable probably due to lack of fermentable sugars.

4.3.2 Bacterial content of fermented pea protein-coconut milk beverage during storage for 21 days /4 $^{\circ}\mathrm{C}$

The viable cell counts of the LAB starter culture bacteria in the fermented pea protein-coconut milk beverage during storage are presented in Figure 4.15. The viable cells of the starter culture in the fermented pea protein-coconut milk beverage increased (p<0.05) from $8.66\pm0.04 \log$ CFU/mL to $8.72\pm0.03 \log$ CFU/mL during the first 14 days of storage, but decreased (p>0.05) to $8.70\pm0.02 \log$ CFU /mL over the last seven days (Appendix E). Probiotic products should contain at least 10^6 CFU /mL or gram at the time of consumption to confer health benefits to the consumer (Kechagia et al. 2013; Hill et al. 2014). Therefore, the cell counts in our cultured beverage met the recommended concentration of probiotic bacteria.



Figure 4.15 Mean VCCs (log CFU /mL) of fermented pea protein-coconut milk beverage during storage (4 °C) for 21 days

Notes: Error bars are means \pm standard deviation; n=3.

Viable cell counts of the starter culture in the fermented beverage during storage may be affected by factors such as type of strains, substrate and storage temperature. Legumes contain complex carbohydrates (fiber, long-lasting starch and oligosaccharide) which contain growth factors and prebiotic components (Miller et al. 2000). Such growth factors and prebiotic

ingredients can promote the growth of the starter culture in the fermented beverage during storage. The decline in VCCs of the fermented beverage during storage was similar to other studies (Mauro & Garcia, 2019; Kazakos et al., 2016). The decrease in cell counts could be to the lack of nutrients after storage for 14 days which affected the continued growth of the microorganisms. It is well-documented that the accumulation of metabolic products such as lactic acid can inhibit the growth or survival of the LAB (Archbold et al., 2010; Costa et al., 2017; Miller et al. 2000). Several studies have shown a decrease in LAB in fermented products during storage (Costa et al., 2017; Santos et al., 2014; Freire et al., 2017). Costa et al. (2017) observed a decrease from 10^6 to 10^5 and 10^4 CFU/g after 28 days of storage for *L. acidophilus* and *Bifidobacterium* spp. cells, respectively, in beverages produced from soya and rice by-products.

4.3.3 Colour of fermented pea protein-coconut milk beverage during storage (4 °C) for 21 days

The colour of the fermented pea protein-coconut milk beverages during storage were measured in Hunter L*, a* and b* values (Figure 4.16). The lightness (L*) increased from 74.48±0.26 (day 1) to 75.76±0.17 (day 14) and then decreased to 72.41±1.37 at day 21 (p<0.05). The redness (a*) of fermented pea protein-coconut milk beverage was stable during the first week of storage, then increased from -0.17±0.11 to 0.38 ± 0.07 from days 7 to 21 (p<0.05). The yellowness (b*) of fermented beverage increased from 4.96±0.31 to 5.17±0.44 in the first 7 days and then decreased to 4.16±0.23 during the last 14 days of refrigerated storage (p<0.05) (Appendix E).





Figure 4.16 Mean colour values of fermented pea protein-coconut milk beverage during storage (4 °C) for 21 days

Notes: Error bars are means \pm standard deviation; n=3. L* represents lightness (0 is black and 100 is diffuse white); a* is the greenness /redness (negative values represent green, positive values represent red) and b* is the blue /yellow (negative values indicate blue, positive values are yellow) (Alqahtani, Aljurais, & Alshaafi, 2012).

In the study by Akin & Ozcan (2017), the yellowness (b*) of the fermented plant protein beverage decreased significantly (p<0.05) during storage for 21days. Hrnjez et al. (2014) also reported similar results during storage of fermented milk inoculated with a mixed LAB-yeast starter culture. The results of these two studies were similar to our results. The lightness of our pea protein-coconut fermented beverage was similar to the results reported by Zare et al. (2013). In our study, the lightness of the fermented beverage increased in the first two weeks during storage (4 °C) which was similar to the probiotic milk supplemented with pea flour reported by Zare et al. (2013). The redness (a*) of our fermented beverage changed during storage (p<0.05), whereas the redness of plant-based fermented milk produced by Akin and Ozcan (2017) was stable. The discrepancies in the results of redness of fermented beverages during storage may be attributed to the different protein sources and protein concentrations used.

4.3.4 Changes in sugar and organic acid levels in fermented pea protein-coconut milk beverage during storage (4 °C) for 21 days

The sucrose concentration in fermented pea protein-coconut milk beverage decreased from $3.65\pm0.03\%$ to $1.68\pm0.01\%$ (p<0.05) (Appendix E), whereas fructose did not change during storage for 21 days /4 °C. (Figure 4.17). The decrease in sucrose levels was expected as the disaccharide is hydrolysed to glucose and fructose moieties by the enzyme invertase, which is metabolised by the fermenting LAB culture (Stadie et al., 2013).



Figure 4.17 Concentrations of sugars and organic acids in fermented pea protein-coconut milk beverage during storage (4 °C) for 21 days

Notes: Error bars are means \pm standard deviation; n=3.

Acetic acid and lactic acid steadily decreased from $2.61\pm0.03\%$ to $2.12\pm0.01\%$ and from $1.41\pm0.04\%$ to $1.32\pm0.04\%$ (w/v) respectively during refrigerated storage for 21 days (p<0.05) (Figure 4.17). Menezes et al. (2018) reported similar reductions of lactic and acetic acids during storage of maize-based beverage stored for 28 days at 4 °C. The decreases in lactic acid observed during storage in our study and previous work may result from the metabolism of lactic acid to pyruvic acid, ethanol and CO₂ (Paucean et al., 2012). The reduction of organic acids, mainly acetic acid, is considered to be a positive factor (Menezes et al., 2018) because at high concentrations acetic acid (>0.34 g /L) may provide off-flavours.

4.3.5 Protein content of fermented pea protein-coconut milk beverage during storage (4 °C) for 21 days

There was no change in protein concentration of the fermented pea protein-coconut milk beverage during storage for 21 days /4 °C (Appendix E). Peng et al. (2009) and Lucey (2001) also reported stable protein content of plant protein beverage products during storage.

4.3.6 Changes in sensory characteristics of fermented pea protein-coconut milk beverage during storage (4 °C) for 21 days

Results of the semi-trained sensory panel (n=15) sensory evaluation of fermented pea proteincoconut milk beverage during storage are shown in Figure 4.18. The scores for appearance, sourness, flavor, mouthfeel and after-taste were changed significantly during 21 days, 4 °C storage (p<0.05). However, mean sensory scores for aroma, sweetness and overall acceptability were stable (p>0.05). The scores for overall acceptability slightly decreased from 6.8 ± 0.40 (day 1) to 6.2 ± 0.20 (day 21) (p>0.05). Thus, the products were slightly like by the panelists on day 21 (Appendix E). The results of the sensory evaluation suggest that although some sensory properties changed during the three weeks cold-storage (4 °C), the fermented pea proteincoconut milk beverage still accepted by the consumers.



Figure 4.18 Mean training group sensory evaluation scores of fermented pea protein-coconut milk beverage during the storage time

Notes: Error bars are means ± standard deviation; n=3; A 9-point hedonic scale with descriptive anchors was used to evaluate each parameter (1= dislike extremely; 9 = like extremely)

The results of the sensory evaluation were similar to those reported by Kilic et al. (1999) who reported no significant effects of storage (4 °C) for 21 days on the sensory attributes of milk yogurt with plant proteins, but the overall acceptability decreased. Awobusuyi (2015) reported improved stability of a non-alcoholic fermented cereal beverage during storage. The samples of the fermented cereal beverage were more accepted at 5 days of storage.

4.3.7 Summary

Phase 3 monitored the change of pH, titratable acidity (T.A.), viable cell counts (VCCs), colour, sugars, organic acid, protein content and sensory characteristics of the fermented pea protein-coconut milk beverage during the 21 days storage (4 °C). The values of pH, T.A., VCCs, color, sugar and organic acid all had significant changes during the storage period (p<0.05). And the beverage product was well-liked by the sensory consumer panelists on using a hedonic rating scale. These results confirmed that although the physical-chemical properties of fermented pea protein-coconut milk beverage changed during the storage period, the beverage still can be store 21 days without loss sensory acceptability characteristics.

5. OVERALL CONCLUSION

The purification methods used in this study was successful in transforming the yellow commercial pea protein powder into a white paste. On a dry basis, the protein concentration of the refined pea protein paste increased by 15% compared with the commercial pea protein powder after the extraction process due to the protein enrichment. The treatment of pea protein-coconut milk containing 3% pea protein produced the most acceptable fermented beverage by a consumer panel. The beverage was characterized by high viable cell counts of the starter culture used and was stable during storage for 21 days at 4 °C. The beany flavour of the pea protein-coconut milk beverage and during storage.

6. RECOMMENDATIONS

The following recommendations are suggested for future research:

The white refined pea protein paste may be used as a source of protein supplement in high protein food products such as pea protein biscuit, pea protein-plant milk beverage and pea protein-dairy yoghurt.

Gas chromatography-mass spectrometry analysis of the organic compounds which are responsible for the bitter and beany flavors is desirable.

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APPENDICES

Product name and Brand	Ingredients	Composition (g)
MRS agar (CM0361), Oxoid	Pepton	10
	Lab-Lemco powder	8
	Yeast extract	4
	Hydrogen phosphate	2
	Sodium acetate 3H2O	5
	Tri-ammonium citrate	2
	Magnesium sulphate 7H2O	0.2
	Manganese sulphate 4H2O	0.05
	Agar	10
M17 agar (CM0785), Oxoid	Tryptone	5
	Soya peptone	5
	Meat digest	5
	Yeast extract	2.5
	Ascorbic acid	0.5
	Magnesium sulphate	0.25
	di-sodium-β-glycerophosphate	19
	Agar	11

Appendix A. Composition of agar media and its ingredient for microbiological analyses
Appendix B. Sensory evaluation questionnaire, participant information sheet and consent form

SENSORY EVALUATION QUESTIONNAIRE

Panelist Number: Date:

Please evaluate the product given to you by selecting ($\sqrt{}$) the attribute that best describes your feelings about the respective property of the product. You will be provided with three samples to taste. Please rinse your mouth with water before and between samples.

Sample Code:

Attribute	Dislike extremely	Dislike very much	Dislike moderately	Dislike slightly	Neither like nor dislike	Like slightly	Like moderately	Like very much	Like extremely
Appearance									
Aroma									
Sourness									
Sweetness									
Flavour									
Mouth feel									
After-taste									
Overall Acceptability									

Comment:

PARTICIPANT INFORMATION SHEET

Study title: Consumer sensory evaluation of fermented coconut beverage containing pea protein

Location: Massey University, Auckland. Researcher: Qu Weidi (Master student) Supervisor: Dr Tony Mutukumira Associate Supervisor: Dr Kay Rutherfurd Ethics committee ref. 4000020456 E-mail: quweidivicky@gmail.com E-mail: <u>A.N.Mutukumira@massey.ac.nz</u> E-mail: k.j.Rutherfurd@massey.ac.nz

Introduction

This study is a part of my research project and may contribute to the development of fermented coconut–pea protein beverage. You are being invited to participate in the evaluation of the sensory characteristics of this beverage. The aim of the sensory evaluation is to determine the level of likeness of the beverages and their acceptance.

Participant involvement

The trial involves tasting three types of coconut–pea protein beverages. Your participation will take around 5 minutes. The three types of beverages you will taste may contain all or some of following ingredients: commercial pea protein powder (Roquette S85F), commercial organic coconut milk (Ceres Organics), commercial start culture (Dupont VEGE 053).

You should not take part if you are allergic or may be affected by the consumption of any of the listed ingredients. In the unlikely event of any adverse reaction, medical assistance will be provided. You may advise one of the researchers of any potentially relevant cultural, religious or ethical beliefs which may prevent you from consuming the foods under consideration.

The information collected in this study will not be linked to any individual's identity and will be only used to complete the research project. Should you wish to receive a summary of the findings once data analysis has been completed, please provide your contact email address or phone number.

You are under no obligation to accept this invitation. If you decide to participate, you have the right to:

- Decline to answer any particular questions;
- Withdraw from the study (at any time);
- Ask any questions about the study at any time during participation;
- Provide information on the understanding that your name will not be used unless you give permission to the researcher;

"This project has been evaluated by peer review and judged to be low risk. Consequently, it has not been reviewed by one of the University's Human Ethics Committees. The researcher(s) named above are responsible for the ethical conduct of this research.

If you have any concerns about the conduct of this research that you wish to raise with someone other than the researcher(s), please contact Professor John O'Neill, Director, Research Ethics, telephone 06 350 5249, email humanethics@massey.ac.nz''.

PARTICIPANT CONSENT FORM

I have read and understood the Participant Information Sheet.

I agree to take part in this project.

I have been given the opportunity to ask questions and I am satisfied with the responses.

I understand that my participation is voluntary and that I am free to withdraw at any time without giving a reason.

Declaration by participant:

I hereby consent to take part in this study.

Participant's name:	Panelist Number:
Signature:	Date:

Appendix C. HPLC data results

Standards	Concentration (%) (w /v)	Mean peak area	Mean retention time (minutes)	
	0.50	689602		
	1.00	1374955		
Sucross	1.50	2050978	0.026	
Sucrose	2.00	2754818	9.020	
	2.50	3414687		
	3.00	3999856		
	0.05	70289		
	0.10	122467		
Emertence	0.15	202530	12 /18	
Fructose	0.20	265223	15.418	
	0.25	315695		
	0.30	390990		
	0.3125	2394658		
	0.6250	4782754		
Lactic acid	1.2500	9433185	17.564	
	2.5000	19781525		
	5.0000	39610808		
	0.3125	2588825		
	0.6250	5156965		
Acetic acid	1.2500	11022641	20.178	
	2.5000	24023412		
	5.0000	46932663		

C.1 HPLC data results for sugar and organic acids standard peak area and retention time

C.2 Standard curves



C.2.2 Organic acid





Appendix D. Raw data

D.1 Raw data phase 1: Commercial pea protein powder purification

D.1.1 Description of colour of refined commercial pea protein powder

Colour	Replication	L*	a*	b*
Commonsial	1	76.76	+3.09	+16.23
commercial pea	2	76.97	+3.09	+16.33
protein powder	3	76.92	+3.06	+16.41
	1	81.49	+0.42	+6.77
Refined pea	2	81.67	+0.30	+6.99
protein paste	3	80.27	+0.33	+6.82

D.1.2 Protein content of refined pea protein

Protein	Replication	Nitrogen %	Protein %
Communicitation	1	12.48	78.00
Commercial pea	2	12.30	76.88
protein powder	3	12.39	77.44
Defining	1	2.77	17.31
Relining pea	2	2.82	17.63
protein paste	3	2.72	16.99
Refining pea	1	14.70	91.87
protein paste	2	15.08	94.24
(dry matter)	3	18.84	92.78

D.2 Raw data phase 2: Development of fermented pea protein-coconut milk beverage

Time (h)	Replication	pH (35 °C)	pH (37 °C)	pH (40 °C)	pH (43 °C)	pH (45 °C)
0	1	6.20	6.20	6.20	6.20	6.20
0	2	6.20	6.20	6.20	6.20	6.20
0	3	6.20	6.20	6.20	6.20	6.20
2	1	6.09	6.03	5.75	5.71	5.62
2	2	6.12	6.05	5.79	5.73	5.66
2	3	6.06	6.01	5.71	5.69	5.58
4	1	5.21	5.15	5.05	4.57	4.55
4	2	5.26	5.21	5.10	4.65	4.60
4	3	5.16	5.09	5.00	4.49	4.50
6	1	4.74	4.66	4.56	4.28	4.25
6	2	4.78	4.71	4.64	4.34	4.29
6	3	4.70	4.61	4.48	4.22	4.21
8	1	4.49	4.39	4.36	4.13	4.08
8	2	4.54	4.42	4.42	4.17	4.13
8	3	4.44	4.36	4.30	4.09	4.03
10	1	4.30	4.25	4.21	4.05	3.98
10	2	4.37	4.31	4.29	4.10	4.05
10	3	4.23	4.19	4.13	4.00	3.92
12	1	4.19	4.13	4.09	3.98	3.92
12	2	4.26	4.18	4.15	4.06	3.97
12	3	4.12	4.08	4.03	3.90	3.87

D.2.1 The pH of coconut milk fermentation by different temperatures

D.2.2 The pH of pea protein paste and coconut milk fermentation by different pea protein paste concentration

Time (h)	Replication	pH (3%)	pH (5%)	pH (7%)	pH (9%)	pH (11%)
0	1	6.20	6.20	6.20	6.20	6.20
0	2	6.20	6.20	6.20	6.20	6.20
0	3	6.20	6.20	6.20	6.20	6.20
2	1	5.76	5.75	5.71	5.66	5.70
2	2	5.79	5.77	5.75	5.68	5.67
2	3	5.73	5.73	5.67	5.64	5.73
4	1	5.08	5.05	5.11	5.15	5.12
4	2	5.13	5.11	5.16	5.18	5.17
4	3	5.03	4.99	5.06	5.12	5.07
6	1	4.53	4.56	4.63	4.65	4.64
6	2	4.57	4.61	4.66	4.71	4.60
6	3	4.49	4.51	4.60	4.59	4.68
8	1	4.30	4.36	4.38	4.42	4.43
8	2	4.35	4.39	4.44	4.46	4.48
8	3	4.25	4.33	4.32	4.38	4.38
10	1	4.06	4.21	4.31	4.30	4.33
10	2	4.46	4.24	4.25	4.34	4.38
10	3	4.11	4.18	4.19	4.26	4.28
12	1	4.12	4.03	4.13	4.19	4.26
12	2	4.05	4.15	4.21	4.24	4.19
12	3	3.97	4.09	4.05	4.14	4.33

Time (h)	Replication	log CFU /mL
0	1	5.48
0	2	5.63
0	3	5.33
2	1	6.96
2	2	7.14
2	3	6.78
4	1	7.70
4	2	7.93
4	3	7.47
6	1	8.08
6	2	8.25
6	3	7.91
8	1	8.63
8	2	8.88
8	3	8.38
10	1	8.54
10	2	8.80
10	3	8.28
12	1	8.45
12	2	8.68
12	3	8.22
14	1	7.89
14	2	7.56
14	3	8.23
16	1	7.36
16	2	7.19
16	3	7.54
18	1	7.08
18	2	7.19
18	3	6.94
20	1	6.98
20	2	7.19
20	3	6.77
22	1	6.82
22	2	7.05
22	3	6.63
24	1	6.88
24	2	6.51
24	3	6.74

D.2.3 Viable cell counts (log CFU /mL) of pea protein paste and coconut milk fermentation for 24 hours

Sample	Replication	Cell count /log CFU /mL
1	1	8.78
1	2	8.99
1	3	8.57
2	1	8.54
2	2	8.22
3	3	8.86
3	1	8.43
3	2	8.87
3	3	7.99
4	1	8.65
4	2	8.98
4	3	8.32
5	1	8.18
5	2	7.96
5	3	8.40
6	1	8.52
6	2	8.67
6	3	8.37
7	1	8.51
7	2	8.87
7	3	8.15
8	1	8.41
8	2	8.61
8	3	8.21
9	1	8.00
9	2	8.28
9	3	7.72

D. 2.4 Viable cell counts (log CFU /mL) for orthogonal test

D.2.5 Sensory evaluation of semi-trained sensory panel (n=18) of the orthogonal test

Replication 1

					Sample				
Panelist	1	2	3	4	5	6	7	8	9
1	8	2	6	5	5	6	7	5	2
2	5	5	4	8	5	8	9	8	3
3	5	7	3	4	5	4	3	7	4
4	7	5	7	5	1	6	9	5	5
5	7	3	3	7	4	3	6	4	7
6	4	8	4	4	4	7	5	4	5
Total score	36	30	27	33	24	34	39	33	26
Average	6.0	5.0	4.5	5.5	4.0	5.7	6.5	5.5	4.3

Replication 2

					Sample				
Panelist	1	2	3	4	5	6	7	8	9
1	7	5	4	8	6	9	8	9	4
2	9	3	6	5	1	7	7	7	5
3	7	8	4	7	4	6	6	4	5
4	6	7	7	5	7	4	7	6	4
5	6	7	3	6	4	7	4	5	6
6	7	6	5	4	2	7	3	6	4
Total score	42	36	29	35	24	40	35	37	28
Average	7.0	6.0	4.8	5.8	4.0	6.7	5.8	6.2	4.7

Replication 3

					Sample				
Panelist	1	2	3	4	5	6	7	8	9
1	8	2	3	7	7	8	8	8	3
2	8	7	6	5	3	6	8	6	4
3	6	6	4	5	3	5	4	5	4
4	7	6	7	7	5	5	8	7	7
5	5	6	4	4	3	5	5	4	6
6	5	6	4	6	3	8	4	5	3
Total score	39	33	28	34	24	37	37	35	27
Average	6.5	5.5	4.7	5.7	4.0	6.2	6.2	5.8	4.5

D.2.6 Consumer sensory evaluation (n=90) of final fermented pea protein-coconut milk beverage

Replication 1

Sample	Panelists	Appearance	Aroma	Sourness	Sweetnes s	Flavour	Mouth feel	After-taste	Overall Acceptabili tv
1	1	7	7	4	4	4	5	6	4
1	2	7	8	5	7	7	6	6	7
1	3	6	6	7	5	5	4	4	6
1	4	5	7	3	2	2	5	2	2
1	5	5	5	6	6	6	6	6	6
1	6	7	5	4	4	4	5	4	4
1	7	8	8	7	6	7	8	8	8
1	8	5	8	6	6	7	6	6	7
1	9	5	5	5	5	5	4	4	5
1	10	8	8	7	6	6	7	7	7
1	11	7	7	5	5	6	6	5	6
1	12	5	4	4	5	5	5	6	5
1	13	7	8	6	4	6	7	7	7
1	14	6	6	6	6	6	6	6	6
1	15	7	7	6	4	5	6	6	6
1	16	8	7	8	8	8	7	7	8
1	17	7	6	7	7	7	7	7	7
1	18	8	7	8	8	8	7	8	8
1	19	6	6	6	4	5	5	5	5
1	20	5	7	2	3	3	3	4	5
1	21	8	6	7	5	8	8	8	8
1	22	5	5	6	5	6	6	6	6
1	23	8	8	6	6	6	6	6	7
1	24	8	8	5	5	5	9	9	7
1	25	6	6	6	6	6	6	6	6
1	26	8	8	6	5	6	7	6	7
1	27	8	8	7	7	7	7	7	8
1	28	7	7	4	3	3	5	5	4
1	29	6	5	6	5	6	6	4	6
1	30	7	8	6	8	8	8	8	8
1	Total	200	201	171	160	173	183	179	186
1	Average	6.7	6.7	5.7	5.3	5.8	6.1	6.0	6.2
	T							1 1	
Sample	Panelists	Appearance	Aroma	Sourness	Sweetness	Flavour	Mouth feel	After-taste	Overall Acceptability
6	1	7	7	5	6	6	7	6	6
6	2	5	7	2	4	1	2	3	2
6	3	6	6	6	6	6	6	6	6
6	4	5	7	2	2	2	3	2	2
6	5	5	7	4	5	4	4	5	5

	÷		÷	÷		÷			÷
6	11	7	5	7	7	7	6	5	7
6	12	5	6	4	5	6	6	6	6
6	13	7	8	4	7	7	7	7	6
6	14	6	6	3	4	3	6	6	5
6	15	7	6	4	5	6	4	6	6
6	16	7	6	5	5	6	5	5	6
6	17	7	5	7	6	6	7	7	6
6	18	8	8	6	5	6	7	6	6
6	19	6	5	3	2	4	4	4	4
6	20	5	8	2	3	5	2	3	3
6	21	8	8	3	4	5	7	6	7
6	22	5	5	6	5	7	7	6	6.5
6	23	8	8	5	5	5	6	5	5
6	24	8	9	7	9	9	9	9	9
6	25	4	6	4	5	4	4	5	4
6	26	6	6	6	5	6	7	6	7
6	27	7	8	6	7	7	8	8	8
6	28	7	5	2	3	2	5	5	2
6	29	6	5	6	6	7	7	6	6
6	30	7	8	7	8	8	8	8	8
6	Total	191	198	142	156	159	165	164	164.5
6	Average	6.4	6.6	4.7	5.2	5.3	5.5	5.5	5.5

Sample	Panelists	Appearance	Aroma	Sourness	Sweetness	Flavour	Mouth feel	After-taste	Overall Acceptability
7	1	7	6	5	6	6	5	6	6
7	2	7	7	4	5	5	5	5	6
7	3	6	6	6	6	6	6	6	5
7	4	5	7	4	4	2	3	2	2
7	5	5	5	3	3	3	3	3	3
7	6	7	7	7	7	7	4	6	7
7	7	8	5	6	5	4	3	3	4
7	8	5	8	4	5	6	5	5	6
7	9	6	5	5	6	5	5	5	5
7	10	7	8	6	6	7	7	8	7
7	11	6	5	6	7	6	6	5	6
7	12	5	5	5	6	7	6	5	7
7	13	8	8	7	6	6	4	4	6
7	14	6	6	3	3	2	1	7	5
7	15	7	6	6	5	4	6	6	6
7	16	7	6	7	6	8	7	5	6
7	17	7	6	6	6	7	7	6	6
7	18	8	7	8	7	7	7	6	7
7	19	6	7	4	4	5	5	5	5
7	20	5	8	3	7	6	6	6	6
7	21	6	5	4	5	6	6	6	5
7	22	4	5	4	5	4	4	5	5
7	23	8	7	4	6	5	2	4	3
7	24	8	8	6	6	6	8	8	8
7	25	6	6	6	6	6	6	6	6
7	26	8	8	3	5	3	3	3	4
7	27	6	5	6	6	6	6	6	6
7	28	7	7	4	3	3	5	5	4
7	29	4	5	5	5	4	4	4	4
7	30	7	8	6	8	8	9	8	8
7	Total	192	192	153	165	160	154	159	164
7	Average	6.4	6.4	5.1	5.5	5.3	5.1	5.3	5.5

Replication 2

Sample	Panelists	Appearance	Aroma	Sourness	Sweetness	Flavour	Mouth feel	After-taste	Overall Acceptability
1	1	7	7	4	4	4	5	6	4
1	2	7	8	5	7	7	6	6	7
1	3	6	6	7	5	5	4	4	6
1	4	5	7	4	2	2	5	2	2

								1	
1	5	5	5	6	6	6	6	6	6
1	6	7	5	4	4	4	5	4	4
1	7	8	8	7	8	7	8	7	8
1	8	6	8	6	6	5	6	6	7
1	9	5	6	5	5	5	4	4	5
1	10	8	8	7	6	6	7	7	7
1	11	7	7	5	5	6	6	5	6
1	12	6	5	6	7	5	5	6	6
1	13	7	8	6	4	6	7	6	7
1	14	6	6	6	6	6	5	6	6
1	15	8	7	6	4	5	5	6	6
1	16	8	7	8	8	6	6	7	8
1	17	7	6	7	7	7	6	7	7
1	18	8	7	6	8	6	6	7	8
1	19	6	6	6	4	5	4	5	5
1	20	6	7	4	5	3	3	4	6
1	20	8	7	7	5	8	8	8	8
1	21	6	5	6	5	6	6	6	6
1	22	8	7	6	6	6	6	6	7
1	23	7	8	7	5	5	9	9	7
1	24	6	6	6	5	5	6	,	6
1	25	0 8	0	0	5	6	0	6	7
1	20	0	0	0	3	0	7	0	7
1	27	7	8	7	1	2	1	/	8
1	28	1	7	5	5	5	3	3	3
1	29	6	5	6	5	6	6	4	6
1	30	/	8	0	8	8	8	8	8
1	Total	203	203	1//	166	16/	1//	1/6	189
1	Average	6.8	6.8	5.9	5.5	5.6	5.9	5.9	6.3
		r							1
Sample	Panelists	Appearance	Aroma	Sourness	Sweetness	Flavour	Mouth feel	After-taste	Overall Acceptability
6	1	7	7	5	6	6	7	6	6
6	2	5	7	2	4	1	2	3	2
6	3	6	6	6	6	6	6	6	6
6	4	5	7	2	2	2	3	2	2
6	5	5	7	4	5	4	4	5	5
6	6	7	5	6	4	4	4	5	4
6	7	8	6	8	7	4	3	3	6
6	8	5	8	5	5	6	6	6	6
6	9	5	6	3	5	4	3	4	3
6	10	7	8	4	6	6	5	5	6
6	11	7	5	7	7	7	6	5	7
6	12	5	6	4	5	6	6	6	6
6	13	7	8	5	7	7	7	7	6
6	14	6	6	3	4	3	6	6	5
6	15	7	6	4	5	6	4	6	6
6	16	7	6	5	5	6	5	5	6
6	17	7	5	7	6	6	7	5	6

6	13	7	8	5	7	7	7	7	6
6	14	6	6	3	4	3	6	6	5
6	15	7	6	4	5	6	4	6	6
6	16	7	6	5	5	6	5	5	6
6	17	7	5	7	6	6	7	5	6
6	18	8	8	6	5	6	7	6	6
6	19	6	5	3	2	4	4	4	4
6	20	5	8	2	3	5	2	3	3
6	21	8	8	3	4	5	7	6	7
6	22	5	5	6	5	7	7	6	6.0
6	23	8	8	5	5	5	6	5	5
6	24	8	9	7	9	9	9	7	9
6	25	4	6	4	5	4	4	5	4
6	26	6	6	6	5	6	7	6	7
6	27	7	8	6	7	7	8	6	7
6	28	7	5	2	3	2	5	5	2
6	29	6	5	6	6	7	7	6	6
6	30	7	8	7	8	8	8	8	8
6	Total	191	198	143	156	159	165	158	162
6	Average	6.4	6.6	4.8	5.2	5.3	5.5	5.3	5.4

Sample	Panelists	Appearance	Aroma	Sourness	Sweetness	Flavour	Mouth feel	After-taste	Overall Acceptability
7	1	7	6	5	6	6	5	6	6
7	2	7	7	4	5	5	5	5	6
7	3	6	6	6	6	6	6	6	5
7	4	5	7	4	4	2	3	2	2
7	5	5	5	3	3	3	3	3	3
7	6	7	7	7	7	7	4	6	7
7	7	8	5	6	5	4	3	3	5
7	8	5	8	4	5	6	5	5	6
7	9	6	5	5	6	5	5	5	5
7	10	7	8	6	6	7	7	8	7
7	11	6	5	6	7	6	6	5	8
7	12	5	5	5	6	7	6	5	7
7	13	8	8	7	6	6	4	4	6
7	14	6	6	3	4	2	1	7	5
7	15	7	6	6	5	4	6	6	6
7	16	7	6	7	6	8	7	5	6
7	17	7	6	6	6	7	7	6	6
7	18	8	7	8	7	7	7	6	7
7	19	6	7	4	5	5	5	5	5
7	20	5	8	3	7	6	6	6	6
7	21	6	5	4	5	6	6	6	5
7	22	4	5	4	5	4	4	5	5
7	23	8	7	4	6	5	2	4	5
7	24	8	8	6	6	6	8	8	8
7	25	6	6	6	6	6	6	6	6
7	26	8	8	3	5	3	3	3	4
7	27	6	5	6	6	6	6	6	6
7	28	7	7	4	4	3	5	5	4
7	29	4	5	5	5	4	4	4	4
7	30	7	8	6	8	8	9	8	8
7	Total	192	192	153	168	160	154	159	169
7	Average	6.4	6.4	5.1	5.6	5.3	5.1	5.3	5.6

Replication 3

Sample	Panelists	Appearance	Aroma	Sourness	Sweetness	Flavour	Mouth feel	After-taste	Overall Acceptability
1	1	7	7	4	4	4	5	6	4
1	2	7	8	5	7	7	6	6	7
1	3	6	6	7	5	5	4	4	6
1	4	5	7	3	2	2	5	2	2
1	5	5	5	6	6	6	6	6	6
1	6	7	5	4	4	4	5	4	4
1	7	8	8	5	6	7	8	8	7
1	8	5	8	6	3	7	6	6	7
1	9	5	5	5	5	7	6	5	5
1	10	8	8	5	6	6	7	7	7
1	11	6	7	5	5	6	6	5	6
1	12	5	4	4	5	5	5	6	5
1	13	7	7	6	4	6	7	7	7
1	14	6	6	6	6	6	6	6	6
1	15	7	7	6	4	5	6	6	6
1	16	7	7	8	8	8	7	7	7
1	17	7	6	7	7	7	7	7	7
1	18	8	7	8	6	8	7	8	8
1	19	6	6	6	4	5	5	5	5
1	20	5	7	2	3	5	5	5	5
1	21	8	6	5	5	8	8	8	8
1	22	5	5	6	5	6	6	6	6
1	23	8	7	6	6	6	6	6	7
1	24	8	8	5	5	5	9	9	7
1	25	5	6	6	6	6	6	6	5
1	26	8	8	6	5	6	7	7	7

1	27	8	8	7	5	7	7	7	8
1	28	7	7	4	3	5	7	5	4
1	29	6	5	6	5	6	6	4	6
1	30	7	8	6	8	8	8	8	8
1	Total	197	199	165	153	179	189	182	183
1	Average	6.6	6.6	5.5	5.1	6.0	6.3	6.1	6.1
				[1		[1	[
Sample	Panelists	Appearance	Aroma	Sourness	Sweetness	Flavour	Mouth feel	After-taste	Overall Acceptability
6	1	7	7	5	6	6	7	6	6
6	2	5	7	2	4	1	2	3	2
6	3	6	6	6	6	6	6	6	6
6	4	5	7	2	2	2	3	2	2
6	5	5	7	4	5	4	4	5	5
6	6	7	5	6	4	4	4	5	4
6	7	8	6	8	7	4	3	3	6
6	8	5	8	5	5	6	6	6	6
6	9	5	6	3	5	4	3	6	4
6	10	7	8	4	6	6	5	5	6
6	11	1	5	/	1	1	6	5	8
6	12	5	6	4	5	6	6	6	6
0	13	6	8	4	1	7	6	6	5
0	14	7	6	3	4	5	0	6	5
6	15	7	6	4	5	6		7	7
6	10	7	5	7	6	6	7	7	6
6	18	8	8	6	5	6	7	6	6
6	19	6	5	3	2	4	4	4	4
6	20	5	8	3	3	5	2	5	3
6	21	8	8	3	4	5	7	6	7
6	22	5	5	6	5	7	7	6	7.0
6	23	8	8	5	5	5	6	5	5
6	24	8	9	6	9	9	9	9	9
6	25	4	6	4	5	4	4	5	4
6	26	6	6	5	5	6	7	6	7
6	27	7	8	6	7	7	8	8	8
6	28	7	5	3	3	2	5	5	2
6	29	6	5	6	6	7	7	6	6
6	30	7	8	7	8	8	8	8	8
6	Total	191	198	141	156	159	165	170	167
6	Average	6.4	6.6	4.7	5.2	5.3	5.5	5.7	5.6
Sample	Panelists	Appearance	Aroma	Sourness	Sweetness	Flavour	Mouth feel	After-taste	Overall Acceptability
7	1	7	6	5	6	6	5	6	6
7	2	7	7	4	5	5	5	5	6
7	3	6	6	6	6	6	6	6	5
7	4	5	7	4	4	2	3	2	2
7	5	5	5	3	3	3	3	3	3
7	6	7	7	7	7	7	4	6	6
7	7	8	5	6	5	4	3	3	4
7	8	5	8	4	5	6	5	5	6
7	9	6	5	5	6	5	5	5	5
7	10	1	8	6	6	1	1	8	5
/	11	6	5	6		6	6	5	6
1	12	5 0	<u>ح</u>	<u>כ</u>	0	 	0	5	
7	13	ð 6	ð 6	2	2	0 2	4	4	5
7	14	7	6	6	5	<u> </u>	6	6	5
7	15	7	6	7	6	*	7	5	6
7	17	7	6	6	5	7	7	6	6
7	18	, 8	7	8	6	7	7	6	7
7	19	6	, 7	4	4	5	5	5	5
7	20	5	8	3	7	6	6	6	6
7	21	6	5	4	5	6	6	6	5

7	22	4	5	4	5	4	4	5	5
7	23	8	7	4	6	5	2	4	3
7	24	8	8	6	6	6	8	8	6
7	25	6	6	6	6	6	6	6	6
7	26	8	8	3	5	3	3	3	4
7	27	6	5	6	5	6	6	6	6
7	28	7	7	4	3	3	5	5	4
7	29	4	5	5	5	4	4	4	4
7	30	7	8	6	8	8	9	8	8
7	Total	192	192	153	162	160	154	159	159
7	Average	6.4	6.4	5.1	5.4	5.3	5.1	5.3	5.3

D.2.7 The pH and titratable acid of fermented pea protein-coconut milk beverage during the fermentation

Time (h)	Replication	pН	T.A. (mL)	T.A. (%)
0	1	6.30	0.55	0.10
0	2	6.09	0.50	0.09
0	3	6.07	0.50	0.09
2	1	6.05	0.65	0.12
2	2	5.93	0.60	0.11
2	3	5.98	0.70	0.13
4	1	5.03	1.20	0.22
4	2	5.21	1.40	0.25
4	3	5.17	1.50	0.27
6	1	4.50	1.70	0.31
6	2	4.59	2.00	0.36
6	3	4.53	1.90	0.34
8	1	4.28	2.90	0.52
8	2	4.31	2.70	0.49
8	3	4.28	3.00	0.54

 $D.2.8\ VCCs\ (log\ CFU\ /mL)$ of fermented pea protein-coconut milk beverage during the fermentation

Time (h)	Replication	Log CFU /mL
0	1	5.54
0	2	5.44
0	3	5.64
2	1	6.99
2	2	6.71
2	3	7.27
4	1	7.85
4	2	7.55
4	3	8.15
6	1	8.18
6	2	8.52
6	3	7.84
8	1	8.61
8	2	8.96
8	3	8.26

Time (h)	Replication	L*	a*	b*
0	1	73.23	0.04	3.01
0	2	73.71	0.03	3.03
0	3	73.25	0.03	2.94
2	1	73.58	0.03	3.15
2	2	73.75	0.02	3.98
2	3	73.46	0.03	3.57
4	1	74.65	0.02	3.96
4	2	73.93	0.01	4.68
4	3	74.06	0.01	3.77
6	1	74.34	-0.01	4.59
6	2	74.27	-0.02	5.08
6	3	74.19	-0.02	4.96
8	1	74.48	-0.03	5.06
8	2	74.73	-0.02	4.61
8	3	74.22	-0.02	5.2

D.2.9 Colour of fermented pea protein-coconut milk beverage during the fermentation

D.2.10 Sugars and organic acids in fermented pea protein-coconut milk beverage during fermentation

Sugars

Time (h)	Replication	Peak area	Sucrose concentration (%) (w /v)
0	1	250250	2.27
0	2	239824	2.17
0	3	240037	2.17
2	1	231999	2.09
2	2	231915	2.09
2	3	221957	1.99
4	1	234406	2.11
4	2	224236	2.01
4	3	224321	2.01
6	1	223234	2.00
6	2	213204	1.90
6	3	213219	1.90
8	1	211535	1.88
8	2	204918	1.82
8	3	203226	1.80

Time (h)	Replication	Peak area	Fructose concentration (%) (w /v)
0	1	137585	0.14
0	2	147936	0.15
0	3	137764	0.14
2	1	166992	0.17
2	2	166131	0.16
2	3	186567	0.18
4	1	215193	0.21
4	2	236206	0.23
4	3	205697	0.20
6	1	255144	0.25
6	2	264005	0.26
6	3	224577	0.22
8	1	293453	0.29
8	2	253956	0.25
8	3	261377	0.26

Organic acids

Time (h)	Replication	Peak area	Lactic acid concentration (%) (w/y)
0	1	25120	0.23
0	2	32916	0.24
0	3	29018	0.24
2	1	121602	0.36
2	2	110391	0.34
2	3	110997	0.34
4	1	344806	0.63
4	2	434095	0.75
4	3	374451	0.67
6	1	624723	0.98
6	2	601136	0.95
6	3	567930	0.91
8	1	753267	1.14
8	2	896269	1.32
8	3	824768	1.23
			A
Time (h)	Replication	Peak area	Acetic acid concentration $\binom{9}{2}$ (w/w)
Time (h)	Replication 1	Peak area	Acetic acid concentration (%) (w /v)
Time (h)	Replication 1 2	Peak area 1302465 1024368	Acetic acid concentration (%) (w /v) 1.86
Time (h) 0 0	Replication 1 2 3	Peak area 1302465 1024368 1213417	Acetic acid concentration (%) (w /v) 1.86 1.56 1.77
Time (h) 0 0 2	Replication 1 2 3 1	Peak area 1302465 1024368 1213417 1371017	Acetic acid concentration (%) (w /v) 1.86 1.56 1.77 1.94
Time (h) 0 0 2 2	Replication 1 2 3 1 2	Peak area 1302465 1024368 1213417 1371017 1224689	Acetic acid concentration (%) (w /v) 1.86 1.56 1.77 1.94 1.78
Time (h) 0 0 2 2 2 2	Replication 1 2 3 1 2 3 1 2 3	Peak area 1302465 1024368 1213417 1371017 1224689 1347853	Acetic acid concentration (%) (w /v) 1.86 1.56 1.77 1.94 1.78 1.91
Time (h) 0 0 2 2 2 2 4	Replication 1 2 3 1 2 3 1 2 3 1 2 3 1 1	Peak area 1302465 1024368 1213417 1371017 1224689 1347853 1971421	Acetic acid concentration (%) (w /v) 1.86 1.56 1.77 1.94 1.78 1.91 2.61
Time (h) 0 0 2 2 2 4 4 4	Replication 1 2 3 1 2 3 1 2 3 1 2 3 1 2	Peak area 1302465 1024368 1213417 1371017 1224689 1347853 1971421 2177164	Acetic acid concentration (%) (w /v) 1.86 1.56 1.77 1.94 1.78 1.91 2.61 2.84
Time (h) 0 0 2 2 2 4 4 4 4	Replication 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3	Peak area 1302465 1024368 1213417 1371017 1224689 1347853 1971421 2177164 2024293	Acetic acid concentration (%) (w /v) 1.86 1.56 1.77 1.94 1.78 1.91 2.61 2.84 2.67
Time (h) 0 0 2 2 2 4 4 4 4 6	Replication 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1	Peak area 1302465 1024368 1213417 1371017 1224689 1347853 1971421 2177164 2024293 3052353	Acetic acid concentration (%) (w /v) 1.86 1.56 1.77 1.94 1.78 1.91 2.61 2.84 2.67 3.81
Time (h) 0 0 2 2 2 4 4 4 4 6 6	Replication 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2	Peak area 1302465 1024368 1213417 1371017 1224689 1347853 1971421 2177164 2024293 3052353 2785200	Acetic acid concentration (%) (w /v) 1.86 1.56 1.77 1.94 1.78 1.91 2.61 2.61 2.84 2.67 3.81 3.51
Time (h) 0 0 2 2 2 4 4 4 4 6 6 6 6	Replication 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3	Peak area 1302465 1024368 1213417 1371017 1224689 1347853 1971421 2177164 2024293 3052353 2785200 2868777	Acetic acid concentration (%) (w /v) 1.86 1.56 1.77 1.94 1.78 1.91 2.61 2.84 2.67 3.81 3.51 3.60
Time (h) 0 0 2 2 2 4 4 4 6 6 6 6 8	Replication 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1	Peak area 1302465 1024368 1213417 1371017 1224689 1347853 1971421 2177164 2024293 3052353 2785200 2868777 3266394	Acetic acid concentration (%) (w /v) 1.86 1.56 1.77 1.94 1.78 1.91 2.61 2.84 2.67 3.81 3.51 3.60 4.05
Time (h) 0 0 2 2 2 4 4 4 6 6 6 6 8 8	Replication 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2	Peak area 1302465 1024368 1213417 1371017 1224689 1347853 1971421 2177164 2024293 3052353 2785200 2868777 3266394 3365445	Acetic acid concentration (%) (w /v) 1.86 1.56 1.77 1.94 1.78 1.91 2.61 2.84 2.67 3.81 3.51 3.60 4.05 4.16

D.2.11 Protein content in fermented pea protein-coconut milk beverage during fermentation

Time (h)	Replication	Nitrogen %	Protein %
0	1	0.36	2.25
0	2	0.35	2.19
0	3	0.36	2.22
2	1	0.35	2.19
2	2	0.36	2.25
2	3	0.37	2.31
4	1	0.37	2.31
4	2	0.36	2.25
4	3	0.36	2.38
6	1	0.37	2.31
6	2	0.37	2.31
6	3	0.38	2.38
8	1	0.37	2.31
8	2	0.39	2.44
8	3	0.38	2.38

D.3 Raw data phase 3: Stability of fermented pea protein-coconut milk beverage during storage at 4 °C for 21 days

D.3.1 pH and titratable acid changes of fermented pea protein-coconut milk beverage during storage (4 $^{\circ}$ C) for 21 days

Store days	Replication	pH	T.A. (mL)	T.A. (%)
	1	4.40	2.90	0.52
1	2	4.46	3.00	0.54
	3	4.43	2.95	0.53
	1	4.41	3.00	0.54
7	2	4.37	3.10	0.56
	3	4.39	3.05	0.55
	1	4.40	3.05	0.55
14	2	4.32	3.15	0.57
	3	4.36	3.10	0.56
	1	4.36	3.05	0.55
21	2	4.40	3.08	0.55
	3	4.38	3.02	0.54

D.3.2 Bacterial content of fermented pea protein-coconut milk beverage during storage (4 $^{\circ}$ C) for 21 days

Store days	Replication	CFU /mL	Log CFU /mL
	1	4.3E+08	8.63
1	2	5.0E+08	8.70
	3	4.6E+08	8.66
	1	5.0E+08	8.70
7	2	5.4E+08	8.73
	3	4.9E+08	8.69
	1	5.0E+08	8.70
14	2	5.3E+08	8.72
	3	5.6E+08	8.75
	1	5.0E+08	8.70
21	2	4.8E+08	8.68
	3	5.1E+08	8.71

D.3.3 Colour of fermented pea protein-coconut milk beverage during storage (4 $^{\circ}$ C) for 21 days

Store days	Replication	L*	a*	b*
	1	74.48	-0.03	+5.06
1	2	74.73	-0.02	+4.61
	3	74.22	-0.02	+5.20
	1	75.39	-0.01	+5.67
7	2	75.98	-0.01	+5.00
	3	74.76	-0.03	+4.84
	1	75.94	+0.32	+4.36
14	2	75.72	+0.31	+4.36
	3	75.61	+0.31	+4.31
	1	70.86	+0.30	+4.39
21	2	73.48	+0.44	+3.94
	3	72.88	+0.39	+4.16

D.3.4 Sugars and organic acid change of fermented pea protein-coconut milk beverage during storage (4 $^{\circ}$ C) for 21 days

Sugars

			Sucrose
Store days	Replication	Peak area	concentration
Store days	replication	I cuit urcu	(%) (w/v)
	1	330159	3.07
1	2	325356	3.02
	3	327758	3.05
	1	307955	2.85
7	2	298087	2.75
	3	303021	2.80
	1	266207	2.43
14	2	264667	2.41
	3	265437	2.42
	1	191145	1.68
21	2	192065	1.69
	3	191605	1.68

Store days	Replication	Peak area	Fructose concentration (%) (w /v)
	1	185789	0.18
1	2	166537	0.16
	3	216163	0.21
	1	197242	0.20
7	2	218658	0.22
	3	257950	0.26
	1	247238	0.25
14	2	275242	0.27
	3	226240	0.22
	1	237909	0.24
21	2	293654	0.29
	3	275782	0.27

Organic acids

Store days	Replication	Peak area	Lactic acid concentration (%) (w /v)
	1	992772	1.44
1	2	934784	1.37
	3	963778	1.41
	1	928068	1.36
7	2	929661	1.37
	3	928865	1.36
	1	932852	1.37
14	2	898516	1.33
	3	915684	1.35
	1	923813	1.36
21	2	867798	1.29
	3	895806	1.32

Store days	Replication	Peak area	Acetic acid concentration (%) (w /v)
	1	1998828	2.64
1	2	1952953	2.59
	3	1975891	2.61
	1	1938087	2.57
7	2	1848903	2.47
	3	1893495	2.52
	1	1859720	2.48
14	2	1825137	2.44
	3	1892429	2.52
	1	1536030	2.12
21	2	1523878	2.11
	3	1529954	2.12

Store days	Replication	Nitrogen %	Protein %
	1	0.33	2.06
1	2	0.32	2.00
	3	0.31	1.94
	1	0.33	2.06
7	2	0.34	2.13
	3	0.35	2.19
	1	0.35	2.19
14	2	0.35	2.19
	3	0.35	2.19
	1	0.35	2.19
21	2	0.34	2.13
	3	0.33	2.06

D.3.5 Protein content of fermented pea protein-coconut milk beverage during storage (4 $^{\circ}$ C) for 21 days

D.3.6 Sensory evaluation of semi-trained sensory panel (n=15) of fermented pea proteincoconut milk beverage during storage (4 $^{\circ}$ C) for 21 days

Replication 1

Store days	Panellist	Appearance	Aroma	Sourness	Sweetness	Flavour	Mouth feel	After-taste	Overall Acceptability
1	1	7	6	6	7	8	7	6	8
1	2	5	6	4	5	5	4	6	6
1	3	6	6	4	5	4	6	7	6
1	4	8	8	7	8	8	7	6	8
1	5	9	9	4	6	5	7	9	6
1	Total	35	35	25	31	30	31	34	34
1	Average	7.0	7.0	5.0	6.2	6.0	6.2	6.8	6.8
7	1	7	7	7	6	6	6	6	7
7	2	6	7	7	8	6	4	5	6
7	3	6	6	5	5	6	6	5	6
7	4	8	6	6	6	7	8	7	7
7	5	7	8	6	7	7	8	7	7
7	Total	34	34	31	32	32	32	30	33
7	Average	6.8	6.8	6.2	6.4	6.4	6.4	6.0	6.6
14	1	6	7	7	7	7	7	6	7
14	2	6	8	4	6	6	6	5	6
14	3	6	5	7	5	6	6	5	6
14	4	7	7	6	7	6	7	7	7
14	5	8	8	7	7	6	6	8	7
14	Total	33	35	31	32	31	32	31	33
14	Average	6.6	7.0	6.2	6.4	6.2	6.4	6.2	6.6
21	1	7	8	7	6	7	8	8	7
21	2	6	7	7	7	7	7	7	7
21	3	5	6	5	6	6	6	6	5
21	4	6	7	5	6	7	7	7	6
21	5	6	7	8	6	7	8	7	6
21	Total	30	35	32	31	34	36	35	31
21	Average	6.0	7.0	6.4	6.2	6.8	7.2	7.0	6.2

Replication 2

Store days	Panellist	Appearance	Aroma	Sourness	Sweetness	Flavour	Mouth feel	After-taste	Overall Acceptability
1	1	7	6	5	7	6	7	6	8
1	2	7	6	4	5	5	4	6	6
1	3	6	6	4	6	4	6	8	6
1	4	8	8	4	8	8	5	6	7
1	5	9	9	4	6	5	7	9	6
1	Total	37	35	21	32	28	29	35	33
1	Average	7.4	7.0	4.2	6.4	5.6	5.8	7.0	6.6
7	1	7	7	7	6	6	6	6	5
7	2	6	7	6	8	6	4	5	6
7	3	6	6	5	7	6	7	8	6
7	4	7	7	6	6	6	8	7	6
7	5	7	8	6	7	7	8	7	7
7	Total	33	35	30	34	31	33	33	30
7	Average	6.6	7.0	6.0	6.8	6.2	6.6	6.6	6.0
14	1	6	7	7	7	7	7	6	7
14	2	6	8	4	6	6	6	5	6
14	3	6	7	7	7	5	6	7	6
14	4	7	7	6	7	6	6	7	6
14	5	8	8	7	7	6	6	8	7
14	Total	33	37	31	34	30	31	33	32
14	Average	6.6	7.4	6.2	6.8	6.0	6.2	6.6	6.4
21	1	4	8	7	6	7	8	8	6
21	2	6	6	7	7	7	7	7	6
21	3	5	6	5	6	8	6	6	5
21	4	6	7	7	6	7	5	5	6
21	5	6	5	8	5	7	7	7	6
21	Total	27	32	34	30	36	33	33	29
21	Average	5.4	6.4	6.8	6.0	7.2	6.6	6.6	5.8

Replication 3

Store days	Panellist	Appearance	Aroma	Sourness	Sweetness	Flavour	Mouth feel	After-taste	Overall Acceptability
1	1	7	6	6	7	8	7	6	8
1	2	5	6	6	5	6	6	6	6
1	3	7	6	7	6	5	6	7	7
1	4	8	8	6	6	7	7	6	8
1	5	6	9	4	6	6	7	8	6
1	Total	33	35	29	30	32	33	33	35
1	Average	6.6	7.0	5.8	6.0	6.4	6.6	6.6	7.0
7	1	7	7	6	6	6	6	6	7
7	2	6	7	7	6	6	4	5	7
7	3	7	6	5	5	7	6	5	8
7	4	8	6	6	6	7	7	5	7
7	5	7	7	6	7	7	8	6	7
7	Total	35	33	30	30	33	31	27	36
7	Average	7.0	6.6	6.0	6.0	6.6	6.2	5.4	7.2
14	1	6	7	7	7	7	7	6	7
14	2	6	6	4	6	6	6	5	6
14	3	6	5	7	5	7	7	5	7
14	4	7	7	6	7	6	7	5	7
14	5	8	8	7	5	6	6	8	7
14	Total	33	33	31	30	32	33	29	34
14	Average	6.6	6.6	6.2	6.0	6.4	6.6	5.8	6.8
21	1	7	8	7	6	7	8	8	7
21	2	6	7	7	7	7	7	7	7
21	3	8	8	5	6	6	8	7	7
21	4	6	8	5	6	5	8	8	6
21	5	6	7	6	7	7	8	7	6
21	Total	33	38	30	32	32	39	37	33
21	Average	6.6	7.6	6.0	6.4	6.4	7.8	7.4	6.6

Appendix E. Statistical output

E.1 Statistical output phase 1: Commercial pea protein powder purification

E.1.1 Statistical analysis of description of colour of refined commercial pea protein powder

						95% Cor	nfidence		
			Mean	Std.	Std.	Interval for Mean		Minimum	Maximum
		IN.	IVIEAL	Deviation	Error	Lower Bound	Upper Bound	winning	Maximum
	Commercial pea protein powder	3	76.8833	.10970	.06333	76.6108	77.1558	76.76	76.97
L	Refined pea protein paste	3	81.1433	.76166	.43975	79.2513	83.0354	80.27	81.67
	Total	6	79.0133	2.38352	.97307	76.5120	81.5147	76.76	81.67
	Commercial pea protein powder	3	3.0800	.01732	.01000	3.0370	3.1230	3.06	3.09
а	Refined pea protein paste	3	.3500	.06245	.03606	.1949	.5051	.30	.42
	Total	6	1.7150	1.49584	.61068	.1452	3.2848	.30	3.09
	Commercial pea protein powder	3	16.3233	.09018	.05207	16.0993	16.5474	16.23	16.41
b	Refined pea protein paste	3	6.8600	.11533	.06658	6.5735	7.1465	6.77	6.99
	Total	6	11.5917	5.18411	2.11640	6.1513	17.0321	6.77	16.41

Descriptives

Test of Homogeneity of Variances

	Levene Statistic	df1	df2	Sig.
L	10.197	1	4	.033
а	5.000	1	4	.089
b	.411	1	4	.556

_	ANOVA							
		Sum of Squares	df	Mean Square	F	Sig.		
	Between Groups	27.221	1	27.221	91.938	.001		
L	Within Groups	1.184	4	.296				
Total	28.406	5						
	Between Groups	11.179	1	11.179	5323.500	.000		
а	Within Groups	.008	4	.002				
	Total	11.188	5					
	Between Groups	134.332	1	134.332	12534.869	.000		
b	Within Groups	.043	4	.011				
	Total	134.375	5					

E.1.2 Statistical analysis of protein content of refined pea protein

Descriptives

			Std.	Std Error	95% Confidence Interval for Mean		Minimum	NA suissuus
	N	Mean	Deviation	Sta. Enoi	Lower Bound	Upper Bound	winninum	Maximum
Commercial pea protein powder	3	77.4400	.56000	.32332	76.0489	78.8311	76.88	78.00
Refined pea protein paste	3	17.3100	.32000	.18475	16.5151	18.1049	16.99	17.63
Refining pea protein paste (dry matter)	3	92.9633	1.19559	.69027	89.9933	95.9333	91.87	94.24
Total	9	62.5711	34.61161	11.53720	35.9663	89.1759	16.99	94.24

Protein concentration (%)

Test of Homogeneity of Variances

Protein concentration	(%)
-----------------------	-----

Levene Statistic	df1	df2	Sig.
2.058	2	6	.209

ANOVA

Protein concentration	(%))
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	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	9580.018	2	4790.009	7786.803	.000
Within Groups	3.691	6	.615		
Total	9583.708	8			

Multiple Comparisons

(I) Different pea protein		Mean	0 1 5	c.	95% Confidence Interval		
		Uifference (I-J)	Std. Error	Sig.	Lower Bound	Upper Bound	
Commercial	Refined pea protein paste	60.13000 [*]	.64039	.000	58.5630	61.6970	
pea protein powder	Refined pea protein paste (dry matter)	-15.52333*	.64039	.000	-17.0903	-13.9564	
Refined pea protein paste	Commercial pea protein powder	-60.13000*	.64039	.000	-61.6970	-58.5630	
	Refined pea protein paste (dry matter)	-75.65333*	.64039	.000	-77.2203	-74.0864	
Refined pea protein paste (dry matter)	Commercial pea protein powder	15.52333 [*]	.64039	.000	13.9564	17.0903	
	Refined pea protein paste	75.65333*	.64039	.000	74.0864	77.2203	

Dependent Variable: Protein concentration (%) LSD

*. The mean difference is significant at the 0.05 level.

E.2 Statistical output phase 2: Development of fermented pea protein-coconut milk beverage

E.2.1 Statistical analysis of the pH of coconut milk fermentation by different temperatures

Between-Subjects Factors				
		Ν		
	0	15		
	2	15		
	4	15		
Time (h)	6	15		
	8	15		
	10	15		
	12	15		
	35	21		
-	37	21		
remperature	40	21		
	43	21		
	45	21		

Levene's Test of Equality of Error Variances^a

Dependent	Variable: pH	

F	df1	df2	Sig.			
.908	34	70	.613			
Tests the null hypothesis that the error variance of the						

dependent variable is equal across groups. a. Design: Intercept + Time + Temperature + Time * Temperature

Tests of Between-Subjects Effects
Dependent Variable: pH

Source	Type III Sum of Squares	df	Mean Square	F	Sig.		
Corrected Model	68.218°	34	2.006	773.967	.000		
Intercept	2470.542	1	2470.542	953000.964	.000		
Time	65.132	6	10.855	4187.389	.000		
Temperature	2.271	4	.568	219.036	.000		
Time * Temperature	.815	24	.034	13.100	.000		
Error	.181	70	.003				
Total	2538.941	105					
Corrected Total	68.400	104					

a. R Squared = .997 (Adjusted R Squared = .996)

Descriptive Statistics Dependent Variable: pH

Time (h)			Std.		
Tempera	ature (°C)	Mean	Deviation	N	
	35	6.2000	0.00000	3	
	37	6.2000	0.00000	3	
0	40	6.2000	0.00000	3	
0	43	6.2000	0.00000	3	
	45	6.2000	0.00000	3	
	Total	6.2000	0.00000	15	
	35	6.0900	.03000	3	
	37	6.0300	.02000	3	
2	40	5.7500	.04000	3	
Z	43	5.7100	.02000	3	
	45	5.6200	.04000	3	
	Total	5.8400	.19380	15	
	35	5.2100	.05000	3	
	37	5.1500	.06000	3	
4	40	5.0500	.05000	3	
4	43	4.5700	.08000	3	
	45	4.5500	.05000	3	
	Total	4.9060	.30142	15	
	35	4.7400	.04000	3	
	37	4.6600	.05000	3	
6	40	4.5600	.08000	3	
0	43	4.2800	.06000	3	
	45	4.2500	.04000	3	
	Total	4.4980	.21119	15	
	35	4.4900	.05000	3	
	37	4.3900	.03000	3	
8	40	4.3600	.06000	3	
0	43	4.1300	.04000	3	
	45	4.0800	.05000	3	
	Total	4.2900	.16818	15	
	35	4.3000	.07000	3	
	37	4.2500	.06000	3	
10	40	4.2100	.08000	3	
10	43	4.0500	.05000	3	
	45	3.9833	.06506	3	
	Total	4.1587	.13726	15	
	35	4.1900	.07000	3	
	37	4.1300	.05000	3	
12	40	4.0900	.06000	3	
	43	3.9800	.08000	3	
	45	3.9200	.05000	3	
	Iotal	4.0620	.11521	15	
	35	5.0314	.78905	21	
	37	4.9729	.80632	21	
Total	40	4.8886	.77358	21	
TULAI	43	4.7029	.84348	21	
	45	4.6576	.85075	21	
	Total	4.8507	.81098	105	

1.	Time	*	Те	m	р	eı	a	tu	re	ì

Dependent variable: pH								
Tim	ne (h)			95% Confidence Interval				
Temp (erature ℃)	Mean	Std. Error	Lower Bound	Upper Bound			
	35	6.200	.029	6.141	6.259			
	37	6.200	.029	6.141	6.259			
0	40	6.200	.029	6.141	6.259			
	43	6.200	.029	6.141	6.259			
	45	6.200	.029	6.141	6.259			
	35	6.090	.029	6.031	6.149			
	37	6.030	.029	5.971	6.089			
2	40	5.750	.029	5.691	5.809			
	43	5.710	.029	5.651	5.769			
	45	5.620	.029	5.561	5.679			
	35	5.210	.029	5.151	5.269			
	37	5.150	.029	5.091	5.209			
4	40	5.050	.029	4.991	5.109			
	43	4.570	.029	4.511	4.629			
	45	4.550	.029	4.491	4.609			
	35	4.740	.029	4.681	4.799			
	37	4.660	.029	4.601	4.719			
6	40	4.560	.029	4.501	4.619			
	43	4.280	.029	4.221	4.339			
	45	4.250	.029	4.191	4.309			
	35	4.490	.029	4.431	4.549			
	37	4.390	.029	4.331	4.449			
8	40	4.360	.029	4.301	4.419			
	43	4.130	.029	4.071	4.189			
	45	4.080	.029	4.021	4.139			
	35	4.300	.029	4.241	4.359			
	37	4.250	.029	4.191	4.309			
10	40	4.210	.029	4.151	4.269			
	43	4.050	.029	3.991	4.109			
	45	3.983	.029	3.925	4.042			
	35	4.190	.029	4.131	4.249			
	37	4.130	.029	4.071	4.189			
12	40	4.090	.029	4.031	4.149			
	43	3.980	.029	3.921	4.039			
	45	3.920	.029	3.861	3.979			

2. Time

Dependent Variable: pH							
			95% Confidence Interval				
Time (h)	Mean	Std. Error	Lower	Upper			
			Bound	Bound			
0	6.200	.013	6.174	6.226			
2	5.840	.013	5.814	5.866			
4	4.906	.013	4.880	4.932			
6	4.498	.013	4.472	4.524			
8	4.290	.013	4.264	4.316			
10	4.159	.013	4.132	4.185			
12	4.062	.013	4.036	4.088			

3. Temperature

Dependent variable: ph							
Temperature (℃)			95% Confidence Interval				
	Mean	Std. Error	Lower	Upper			
			Bound	Bound			
35	5.031	.011	5.009	5.054			
37	4.973	.011	4.951	4.995			
40	4.889	.011	4.866	4.911			
43	4.703	.011	4.681	4.725			
45	4.658	.011	4.635	4.680			

Multiple Comparisons

Dependent Variable: pH LSD

200								
(I) Temperature (℃)		Moon			95% Confidence Interval			
		Difference (I-J)	Std. Error	Std. Error Sig.		Upper Bound		
	37	.0586*	.01571	.000	.0272	.0899		
35	40	.1429*	.01571	.000	.1115	.1742		
	43	.3286*	.01571	.000	.2972	.3599		
	45	.3738 [*]	.01571	.000	.3425	.4051		
	35	0586*	.01571	.000	0899	0272		
27	40	.0843*	.01571	.000	.0529	.1156		
37	43	.2700*	.01571	.000	.2387	.3013		
	45	.3152 [*]	.01571	.000	.2839	.3466		
	35	1429*	.01571	.000	1742	1115		
40	37	0843*	.01571	.000	1156	0529		
40	43	.1857*	.01571	.000	.1544	.2171		
	45	.2310 [*]	.01571	.000	.1996	.2623		
	35	3286*	.01571	.000	3599	2972		
12	37	2700 [*]	.01571	.000	3013	2387		
43	40	1857*	.01571	.000	2171	1544		
	45	.0452*	.01571	.005	.0139	.0766		
	35	3738 [*]	.01571	.000	4051	3425		
45	37	3152 [*]	.01571	.000	3466	2839		
45	40	2310 [*]	.01571	.000	2623	1996		
	43	- 0452*	01571	005	- 0766	- 0139		

Based on observed means.

The error term is Mean Square (Error) = .003.

 $\star.$ The mean difference is significant at the .05 level.

Multiple Comparisons

Dependent Variable: pH LSD

(I) Time (h)		Mean			95% Confidence Interval		
		Difference (I-J)	Std. Error	Sig.	Lower Bound	Upper Bound	
	2	.3600*	.01859	.000	.3229	.3971	
	4	1.2940*	.01859	.000	1.2569	1.3311	
0	6	1.7020*	.01859	.000	1.6649	1.7391	
0	8	1.9100*	.01859	.000	1.8729	1.9471	
	10	2.0413*	.01859	.000	2.0043	2.0784	
	12	2.1380*	.01859	.000	2.1009	2.1751	
	0	3600*	.01859	.000	3971	3229	
	4	.9340*	.01859	.000	.8969	.9711	
2	6	1.3420*	.01859	.000	1.3049	1.3791	
2	8	1.5500*	.01859	.000	1.5129	1.5871	
	10	1.6813*	.01859	.000	1.6443	1.7184	
	12	1.7780*	.01859	.000	1.7409	1.8151	
	0	-1.2940*	.01859	.000	-1.3311	-1.2569	
	2	9340 [*]	.01859	.000	9711	8969	
1	6	.4080*	.01859	.000	.3709	.4451	
4	8	.6160*	.01859	.000	.5789	.6531	
	10	.7473 [*]	.01859	.000	.7103	.7844	
	12	.8440 [*]	.01859	.000	.8069	.8811	
	0	-1.7020 [*]	.01859	.000	-1.7391	-1.6649	
	2	-1.3420 [*]	.01859	.000	-1.3791	-1.3049	
6	4	4080*	.01859	.000	4451	3709	
0	8	.2080*	.01859	.000	.1709	.2451	
	10	.3393 [*]	.01859	.000	.3023	.3764	
	12	.4360*	.01859	.000	.3989	.4731	
	0	-1.9100 [*]	.01859	.000	-1.9471	-1.8729	
	2	-1.5500*	.01859	.000	-1.5871	-1.5129	
8	4	6160*	.01859	.000	6531	5789	
Ŭ	6	2080*	.01859	.000	2451	1709	
	10	.1313*	.01859	.000	.0943	.1684	
	12	.2280 [*]	.01859	.000	.1909	.2651	
	0	-2.0413*	.01859	.000	-2.0784	-2.0043	
	2	-1.6813*	.01859	.000	-1.7184	-1.6443	
10	4	7473*	.01859	.000	7844	7103	
	6	3393*	.01859	.000	3764	3023	
	8	1313*	.01859	.000	1684	0943	
	12	.0967*	.01859	.000	.0596	.1337	
	0	-2.1380°	.01859	.000	-2.1751	-2.1009	
	2	-1.7780 [*]	.01859	.000	-1.8151	-1.7409	
12	4	8440 [*]	.01859	.000	8811	8069	
	6	4360*	.01859	.000	4731	3989	
	8	2280*	.01859	.000	2651	1909	
	10	0967*	.01859	.000	1337	0596	

Based on observed means.

The error term is Mean Square (Error) = .003.

 $\star.$ The mean difference is significant at the .05 level.





Between-Subjects Factors						
		Ν				
	0	15				
	2	15				
	4	15				
Time (h)	6	15				
	8	15				
	10	15				
	12	15				
	3	21				
	5	21				
Concentration	7	21				
(70)	9	21				
	11	21				

Tests of Between-Subjects Effects

		Dependent V	anabic. pri		
Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	56.303ª	34	1.656	496.933	.000
Intercept	2535.968	1	2535.968	761007.832	.000
Time	56.079	6	9.347	2804.771	.000
Concentration	.092	4	.023	6.897	.000
Time * Concentration	.132	24	.005	1.646	.056
Error	.233	70	.003		
Total	2592.504	105			
Corrected Total	56.536	104			

a. R Squared = .996 (Adjusted R Squared = .994)

	Dependent			
Time (h)	Concentration (%)	Mean	Std. Deviation	N
	3	6.2000	0.00000	3
	5	6.2000	0.00000	3
0	7	6.2000	0.00000	3
0	9	6.2000	0.00000	3
	11	6.2000	0.00000	3
	Total	6.2000	0.00000	15
	3	5.7600	.03000	3
	5	5.7500	.02000	3
0	7	5.7100	.04000	3
2	9	5.6600	.02000	3
	11	5.7000	.03000	3
	Total	5.7160	.04469	15
	3	5.0800	.05000	3
	5	5.0500	.06000	3
	7	5.1100	.05000	3
4	9	5.1500	.03000	3
	11	5.1200	.05000	3
	Total	5.1020	.05454	15
	3	4.5300	.04000	3
	5	4.5600	.05000	3
<u>_</u>	7	4.6300	.03000	3
6	9	4.6500	.06000	3
	11	4.6400	.04000	3
	Total	4.6020	.06259	15
	3	4.3000	.05000	3
	5	4.3600	.03000	3
0	7	4.3800	.06000	3
ð	9	4.4200	.04000	3
	11	4.4300	.05000	3
	Total	4.3780	.06259	15
	3	4.2100	.21794	3
	5	4.2100	.03000	3
10	7	4.2500	.06000	3
10	9	4.3000	.04000	3
	11	4.3300	.05000	3
	Total	4.2600	.10247	15
	3	4.0467	.07506	3
	5	4.0900	.06000	3
10	7	4.1300	.08000	3
12	9	4.1900	.05000	3
	11	4.2600	.07000	3
	Total	4.1433	.09656	15
	3	4.8752	.79366	21
	5	4.8886	.77280	21
	7	4.9157	.75096	21
Total	9	4,9386	72289	21
	- 11	4 95/3	71317	21
	Total	4.0145	72720	105
	rotal	4.9145	.13130	102

Descriptive Statistics Dependent Variable: pH

Levene's Test of Equality of Error Variancesa Dependent Variable: pH

		Bependent va	
F	df1	df2	Sig.
2.822	34	70	.000

Tests the null hypothesis that the error variance of the dependent variable is equal across groups.

a. Design: Intercept + Time + Concentration + Time * Concentration

Dependent Variable: pH								
			95% Confidence Interval					
Time (h)	Mean	Std. Error	Lower	Upper				
			Bound	Bound				
0	6.200	.015	6.170	6.230				
2	5.716	.015	5.686	5.746				
4	5.102	.015	5.072	5.132				
6	4.602	.015	4.572	4.632				
8	4.378	.015	4.348	4.408				
10	4.260	.015	4.230	4.290				
12	4.143	.015	4.114	4.173				

1. Time Dependent Variable: r

2. Time * Concentration Dependent Variable: pH

Time (h)				95% Confide	ence Interval
Concentration (%)		Mean	Std. Error	Lower Bound	Upper Bound
	3	6.200	.033	6.134	6.266
	5	6.200	.033	6.134	6.266
0	7	6.200	.033	6.134	6.266
	9	6.200	.033	6.134	6.266
	11	6.200	.033	6.134	6.266
	3	5.760	.033	5.694	5.826
	5	5.750	.033	5.684	5.816
2	7	5.710	.033	5.644	5.776
	9	5.660	.033	5.594	5.726
	11	5.700	.033	5.634	5.766
	3	5.080	.033	5.014	5.146
	5	5.050	.033	4.984	5.116
4	7	5.110	.033	5.044	5.176
	9	5.150	.033	5.084	5.216
	11	5.120	.033	5.054	5.186
	3	4.530	.033	4.464	4.596
	5	4.560	.033	4.494	4.626
6	7	4.630	.033	4.564	4.696
	9	4.650	.033	4.584	4.716
	11	4.640	.033	4.574	4.706
	3	4.300	.033	4.234	4.366
	5	4.360	.033	4.294	4.426
8	7	4.380	.033	4.314	4.446
	9	4.420	.033	4.354	4.486
	11	4.430	.033	4.364	4.496
	3	4.210	.033	4.144	4.276
	5	4.210	.033	4.144	4.276
10	7	4.250	.033	4.184	4.316
	9	4.300	.033	4.234	4.366
	11	4.330	.033	4.264	4.396
	3	4.047	.033	3.980	4.113
	5	4.090	.033	4.024	4.156
12	7	4.130	.033	4.064	4.196
	9	4.190	.033	4.124	4.256
	11	4.260	.033	4.194	4.326

3. Concentration Dependent Variable: pH

Dependent variable, pri							
Concentration			95% Confidence Interval				
Concentration	Mean	Std. Error	Lower	Upper			
(%)			Bound	Bound			
3	4.875	.013	4.850	4.900			
5	4.889	.013	4.863	4.914			
7	4.916	.013	4.891	4.941			
9	4.939	.013	4.913	4.964			
11	4.954	.013	4.929	4.979			

Multiple Comparisons

Dependent Variable: pH LSD

I			LJD			
(I) Time (h)		Mean Difference		C.	95% Confidence Interval	
		(I-J)	Sta. Error	Sig.	Lower Bound	Upper Bound
	2	.4840*	.02108	.000	.4420	.5260
	4	1.0980*	.02108	.000	1.0560	1.1400
0	6	1.5980 [*]	.02108	.000	1.5560	1.6400
0	8	1.8220 [*]	.02108	.000	1.7800	1.8640
	10	1.9400 [*]	.02108	.000	1.8980	1.9820
	12	2.0567*	.02108	.000	2.0146	2.0987
	0	4840*	.02108	.000	5260	4420
	4	.6140*	.02108	.000	.5720	.6560
~	6	1.1140*	.02108	.000	1.0720	1.1560
2	8	1.3380*	.02108	.000	1.2960	1.3800
	10	1.4560 [*]	.02108	.000	1.4140	1.4980
	12	1.5727 [*]	.02108	.000	1.5306	1.6147
	0	-1.0980*	.02108	.000	-1.1400	-1.0560
	2	6140 [*]	.02108	.000	6560	5720
4	6	.5000*	.02108	.000	.4580	.5420
4	8	.7240*	.02108	.000	.6820	.7660
	10	.8420*	.02108	.000	.8000	.8840
	12	.9587*	.02108	.000	.9166	1.0007
	0	-1.5980*	.02108	.000	-1.6400	-1.5560
	2	-1.1140*	.02108	.000	-1.1560	-1.0720
C	4	5000*	.02108	.000	5420	4580
0	8	.2240*	.02108	.000	.1820	.2660
	10	.3420*	.02108	.000	.3000	.3840
	12	.4587*	.02108	.000	.4166	.5007
	0	-1.8220*	.02108	.000	-1.8640	-1.7800
	2	-1.3380*	.02108	.000	-1.3800	-1.2960
0	4	7240 [*]	.02108	.000	7660	6820
0	6	2240 [*]	.02108	.000	2660	1820
	10	.1180*	.02108	.000	.0760	.1600
	12	.2347*	.02108	.000	.1926	.2767
	0	-1.9400*	.02108	.000	-1.9820	-1.8980
	2	-1.4560*	.02108	.000	-1.4980	-1.4140
10	4	8420*	.02108	.000	8840	8000
10	6	3420 [*]	.02108	.000	3840	3000
	8	1180 [*]	.02108	.000	1600	0760
	12	.1167*	.02108	.000	.0746	.1587
1	0	-2.0567*	.02108	.000	-2.0987	-2.0146
	2	-1.5727*	.02108	.000	-1.6147	-1.5306
12	4	9587*	.02108	.000	-1.0007	9166
12	6	4587*	.02108	.000	5007	4166
1	8	2347*	.02108	.000	2767	1926
	10	1167*	.02108	.000	1587	0746

Based on observed means.

The error term is Mean Square (Error) = .003. *. The mean difference is significant at the .05 level.

Multiple Comparisons

Dependent Variable: pH

	(I)	Moon			95% Confide	ence Interval		
Concentration (%)		Difference (I-J)	Std. Error	Std. Error Sig.		Upper Bound		
	5	0133	.01781	.457	0489	.0222		
2	7	0405*	.01781	.026	0760	0049		
3	9	0633*	.01781	.001	0989	0278		
	11	0790 [*]	.01781	.000	1146	0435		
	3	.0133	.01781	.457	0222	.0489		
Б	7	0271	.01781	.132	0627	.0084		
5	9	0500*	.01781	.006	0855	0145		
	11	0657*	.01781	.000	1012	0302		
	3	.0405*	.01781	.026	.0049	.0760		
7	5	.0271	.01781	.132	0084	.0627		
í í	9	0229	.01781	.204	0584	.0127		
	11	0386*	.01781	.034	0741	0030		
	3	.0633 [*]	.01781	.001	.0278	.0989		
q	5	.0500*	.01781	.006	.0145	.0855		
5	7	.0229	.01781	.204	0127	.0584		
	11	0157	.01781	.381	0512	.0198		
	3	.0790*	.01781	.000	.0435	.1146		
11	5	.0657*	.01781	.000	.0302	.1012		
11	7	.0386*	.01781	.034	.0030	.0741		
	9	0157	01781	381	- 0198	0512		

Based on observed means.

The error term is Mean Square (Error) = .003.

*. The mean difference is significant at the .05 level.



Estimated Marginal Means of pH

Error bars: 95% CI

E.2.3 Statistical analysis of viable cell counts (log CFU /mL) of pea protein paste and coconut milk fermentation for 24 hours

					95% Confiden	ce Interval for		
Time (h)	N	Mean	Std.	Std. Error	Mean		Minimum	Maximum
Time (n)		Weath	Deviation		Lower Bound	Upper Bound		Waximam
0	3	5.4800	.15000	.08660	5.1074	5.8526	5.33	5.63
2	3	6.9600	.18000	.10392	6.5129	7.4071	6.78	7.14
4	3	7.7000	.23000	.13279	7.1286	8.2714	7.47	7.93
6	3	8.0800	.17000	.09815	7.6577	8.5023	7.91	8.25
8	3	8.6300	.25000	.14434	8.0090	9.2510	8.38	8.88
10	3	8.5400	.26000	.15011	7.8941	9.1859	8.28	8.80
12	3	8.4500	.23000	.13279	7.8786	9.0214	8.22	8.68
14	3	7.8933	.33501	.19342	7.0611	8.7256	7.56	8.23
16	3	7.3633	.17502	.10105	6.9286	7.7981	7.19	7.54
18	3	7.0700	.12530	.07234	6.7587	7.3813	6.94	7.19
20	3	6.9800	.21000	.12124	6.4583	7.5017	6.77	7.19
22	3	6.8333	.21032	.12143	6.3109	7.3558	6.63	7.05
24	3	6.7100	.18682	.10786	6.2459	7.1741	6.51	6.88
Total	39	7.4377	.89106	.14268	7.1488	7.7265	5.33	8.88

Descriptives

Test of Homogeneity of Variances

log CFU /mL

Levene df1 Statistic		df2	Sig.	
.259	12	26	.991	

log CFU /mL

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	28.968	12	2.414	52.174	.000
Within Groups	1.203	26	.046		
Total	30.171	38			

Multiple Comparisons

Dependent Variable: log CFU /mL

LSD									
(I) Time (h)		Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval				
					Lower Bound	Upper Bound			
0	2	-1.48000*	.17563	.000	-1.8410	-1.1190			
	4	-2.22000*	.17563	.000	-2.5810	-1.8590			
	6	-2.60000*	.17563	.000	-2.9610	-2.2390			
	8	-3.15000*	.17563	.000	-3.5110	-2.7890			
	10	-3.06000*	.17563	.000	-3.4210	-2.6990			
	12	-2.97000*	.17563	.000	-3.3310	-2.6090			
	14	-2.41333*	.17563	.000	-2.7743	-2.0523			
	16	-1.88333*	.17563	.000	-2.2443	-1.5223			
	18	-1.59000*	.17563	.000	-1.9510	-1.2290			
	20	-1.50000*	.17563	.000	-1.8610	-1.1390			
	22	-1.35333*	.17563	.000	-1.7143	9923			
	24	-1.23000*	.17563	.000	-1.5910	8690			
	0	1.48000*	.17563	.000	1.1190	1.8410			
	4	74000 [*]	.17563	.000	-1.1010	3790			
	6	-1.12000*	.17563	.000	-1.4810	7590			
	8	-1.67000*	.17563	.000	-2.0310	-1.3090			
2	10	-1.58000*	.17563	.000	-1.9410	-1.2190			
	12	-1.49000*	.17563	.000	-1.8510	-1.1290			
	14	93333 [*]	.17563	.000	-1.2943	5723			
	16	40333*	.17563	.030	7643	0423			
	18	11000	.17563	.537	4710	.2510			
	20	02000	.17563	.910	3810	.3410			
	22	.12667	.17563	.477	2343	.4877			
	24	.25000	.17563	.166	1110	.6110			
	0	2.22000*	.17563	.000	1.8590	2.5810			
4	2	.74000*	.17563	.000	.3790	1.1010			
	6	38000*	.17563	.040	7410	0190			
	8	93000*	.17563	.000	-1.2910	5690			
	10	84000*	.17563	.000	-1.2010	4790			
	12	75000*	.17563	.000	-1.1110	3890			
	14	19333	.17563	.281	5543	.1677			
	16	.33667	.17563	.066	0243	.6977			
	18	.63000*	.17563	.001	.2690	.9910			
	20	.72000*	.17563	.000	.3590	1.0810			
	22	.86667*	.17563	.000	.5057	1.2277			
	24	.99000*	.17563	.000	.6290	1.3510			
	0	2.60000*	.17563	.000	2.2390	2.9610			
	2	1.12000*	.17563	.000	.7590	1.4810			
	4	.38000*	.17563	.040	.0190	.7410			
	8	55000*	.17563	.004	9110	1890			
	10	46000*	.17563	.015	8210	0990			
	12	37000*	.17563	.045	7310	0090			
	14	.18667	.17563	.298	1743	.5477			
	16	.71667*	.17563	.000	.3557	1.0777			
	18	1.01000*	.17563	.000	.6490	1.3710			
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	20	1.10000*	.17563	.000	.7390	1.4610			
	22	1.24667*	.17563	.000	.8857	1.6077			
	24	1.37000*	.17563	.000	1.0090	1.7310			
	0	3.15000*	.17563	.000	2.7890	3.5110			
	2	1.67000*	.17563	.000	1.3090	2.0310			
	4	.93000*	.17563	.000	.5690	1.2910			
	6	.55000*	.17563	.004	.1890	.9110			
	10	.09000	.17563	.613	2710	.4510			
	12	.18000	.17563	.315	1810	.5410			
8	14	.73667*	.17563	.000	.3757	1.0977			
	16	1.26667*	.17563	.000	.9057	1.6277			
	18	1.56000*	.17563	.000	1.1990	1.9210			
	20	1.65000*	.17563	.000	1.2890	2.0110			
	22	1.79667*	.17563	.000	1.4357	2.1577			
	24	1.92000*	.17563	.000	1.5590	2.2810			
	0	3.06000*	.17563	.000	2.6990	3.4210			
	2	1.58000*	.17563	.000	1.2190	1.9410			
	4	.84000*	.17563	.000	.4790	1.2010			
	6	.46000*	.17563	.015	.0990	.8210			
	8	09000	.17563	.613	4510	.2710			
10	12	.09000	.17563	.613	2710	.4510			
10	14	.64667*	.17563	.001	.2857	1.0077			
	16	1.17667*	.17563	.000	.8157	1.5377			
	18	1.47000*	.17563	.000	1.1090	1.8310			
	20	1.56000*	.17563	.000	1.1990	1.9210			
	22	1.70667*	.17563	.000	1.3457	2.0677			
	24	1.83000*	.17563	.000	1.4690	2.1910			
	0	2.97000*	.17563	.000	2.6090	3.3310			
	2	1.49000*	.17563	.000	1.1290	1.8510			
	4	.75000*	.17563	.000	.3890	1.1110			
	6	.37000*	.17563	.045	.0090	.7310			
	8	18000	.17563	.315	5410	.1810			
12	10	09000	.17563	.613	4510	.2710			
12	14	.55667*	.17563	.004	.1957	.9177			
	16	1.08667*	.17563	.000	.7257	1.4477			
	18	1.38000*	.17563	.000	1.0190	1.7410			
	20	1.47000*	.17563	.000	1.1090	1.8310			
	22	1.61667*	.17563	.000	1.2557	1.9777			
	24	1.74000*	.17563	.000	1.3790	2.1010			
	0	2.41333*	.17563	.000	2.0523	2.7743			
	2	.93333 [*]	.17563	.000	.5723	1.2943			
	4	.19333	.17563	.281	1677	.5543			
	6	18667	.17563	.298	5477	.1743			
14	8	73667*	.17563	.000	-1.0977	3757			
	10	64667*	.17563	.001	-1.0077	2857			
	12	55667*	.17563	.004	9177	1957			
	16	.53000*	.17563	.006	.1690	.8910			
l	18	.82333 [*]	.17563	.000	.4623	1.1843			

	20	.91333 [*]	.17563	.000	.5523	1.2743
	22	1.06000*	.17563	.000	.6990	1.4210
	24	1.18333*	.17563	.000	.8223	1.5443
	0	1.88333*	.17563	.000	1.5223	2.2443
	2	.40333*	.17563	.030	.0423	.7643
	4	33667	.17563	.066	6977	.0243
	6	71667 [*]	.17563	.000	-1.0777	3557
	8	-1.26667*	.17563	.000	-1.6277	9057
10	10	-1.17667*	.17563	.000	-1.5377	8157
16	12	-1.08667*	.17563	.000	-1.4477	7257
	14	53000*	.17563	.006	8910	1690
	18	.29333	.17563	.107	0677	.6543
	20	.38333*	.17563	.038	.0223	.7443
	22	.53000*	.17563	.006	.1690	.8910
	24	.65333*	.17563	.001	.2923	1.0143
	0	1.59000*	.17563	.000	1.2290	1.9510
	2	.11000	.17563	.537	2510	.4710
	4	63000 [*]	.17563	.001	9910	2690
	6	-1.01000*	.17563	.000	-1.3710	6490
	8	-1.56000*	.17563	.000	-1.9210	-1.1990
	10	-1.47000*	.17563	.000	-1.8310	-1.1090
18	12	-1.38000*	.17563	.000	-1.7410	-1.0190
	14	82333 [*]	.17563	.000	-1.1843	4623
	16	29333	.17563	.107	6543	.0677
	20	.09000	.17563	.613	2710	.4510
	22	.23667	.17563	.189	1243	.5977
	24	.36000	.17563	.051	0010	.7210
	0	1.50000*	.17563	.000	1.1390	1.8610
	2	.02000	.17563	.910	3410	.3810
	4	72000*	.17563	.000	-1.0810	3590
	6	-1.10000*	.17563	.000	-1.4610	7390
	8	-1.65000*	.17563	.000	-2.0110	-1.2890
00	10	-1.56000*	.17563	.000	-1.9210	-1.1990
20	12	-1.47000*	.17563	.000	-1.8310	-1.1090
	14	91333 [*]	.17563	.000	-1.2743	5523
	16	38333*	.17563	.038	7443	0223
	18	09000	.17563	.613	4510	.2710
	22	.14667	.17563	.411	2143	.5077
	24	.27000	.17563	.136	0910	.6310
	0	1.35333*	.17563	.000	.9923	1.7143
	2	12667	.17563	.477	4877	.2343
	4	86667*	.17563	.000	-1.2277	5057
	6	-1.24667*	.17563	.000	-1.6077	8857
22	8	-1.79667*	.17563	.000	-2.1577	-1.4357
22	10	-1.70667*	.17563	.000	-2.0677	-1.3457
1	12	-1.61667*	.17563	.000	-1.9777	-1.2557
	14	-1.06000*	.17563	.000	-1.4210	6990
1	16	53000*	.17563	.006	8910	1690
	18	23667	.17563	.189	5977	.1243

	20	14667	.17563	.411	5077	.2143
	24	.12333	.17563	.489	2377	.4843
	0	1.23000*	.17563	.000	.8690	1.5910
	2	25000	.17563	.166	6110	.1110
	4	99000*	.17563	.000	-1.3510	6290
	6	-1.37000*	.17563	.000	-1.7310	-1.0090
	8	-1.92000*	.17563	.000	-2.2810	-1.5590
24	10	-1.83000*	.17563	.000	-2.1910	-1.4690
24	12	-1.74000*	.17563	.000	-2.1010	-1.3790
	14	-1.18333*	.17563	.000	-1.5443	8223
	16	65333*	.17563	.001	-1.0143	2923
	18	36000	.17563	.051	7210	.0010
	20	27000	.17563	.136	6310	.0910
	22	12333	.17563	.489	4843	.2377

E.2.4 Statistical analysis of viable cell counts (log CFU /mL) for orthogonal test

Cell count /log CFU /mL										
Experimental number	Ν	Mean	Std.	Std. Error	95% Confiden Me	ce Interval for ean	Minimum	Maximum		
			Deviation		Lower Bound	Upper Bound				
1	3	8.7800	.21000	.12124	8.2583	9.3017	8.57	8.99		
2	3	8.5400	.32000	.18475	7.7451	9.3349	8.22	8.86		
3	3	8.4300	.44000	.25403	7.3370	9.5230	7.99	8.87		
4	3	8.6500	.33000	.19053	7.8302	9.4698	8.32	8.98		
5	3	8.1800	.22000	.12702	7.6335	8.7265	7.96	8.40		
6	3	8.5200	.15000	.08660	8.1474	8.8926	8.37	8.67		
7	3	8.5100	.36000	.20785	7.6157	9.4043	8.15	8.87		
8	3	8.4100	.20000	.11547	7.9132	8.9068	8.21	8.61		
9	3	8.0000	.28000	.16166	7.3044	8.6956	7.72	8.28		
Total	27	8.4467	.33191	.06388	8.3154	8.5780	7.72	8.99		

Descriptives

Test of Homogeneity of Variances

Cell count /log CFU /mL

Levene Statistic	df1	df2	Sig.
.398	8	18	.907

ANOVA

Cell count /log CFU /mL							
	Sum of Squares	df	Mean Square	F	Sig.		
Between Groups	1.328	8	.166	1.946	.115		
Within Groups	1.536	18	.085				
Total	2.864	26					

Multiple Comparisons

Dependent Variable: Cell count /log CFU /mL

	(I)	Mean			95% Confide	ence Interval
Exper nur	imental mber	Difference (I-J)	Std. Error	Sig.	Lower Bound	Upper Bound
	2	.24000	.23850	.328	2611	.7411
	3	.35000	.23850	.159	1511	.8511
	4	.13000	.23850	.592	3711	.6311
1	5	.60000*	.23850	.022	.0989	1.1011
1	6	.26000	.23850	.290	2411	.7611
	7	.27000	.23850	.272	2311	.7711
	8	.37000	.23850	.138	1311	.8711
	9	.78000 [*]	.23850	.004	.2789	1.2811
	1	24000	.23850	.328	7411	.2611
	3	.11000	.23850	.650	3911	.6111
	4	11000	.23850	.650	6111	.3911
	5	.36000	.23850	.149	1411	.8611
2	6	.02000	.23850	.934	4811	.5211
	7	.03000	.23850	.901	4711	.5311
	8	.13000	.23850	.592	3711	.6311
	9	.54000 [*]	.23850	.036	.0389	1.0411
	1	35000	.23850	.159	8511	.1511
	2	11000	.23850	.650	6111	.3911
	4	22000	.23850	.369	7211	.2811
	5	.25000	.23850	.308	2511	.7511
3	6	09000	.23850	.710	5911	.4111
	7	08000	.23850	.741	5811	.4211
	8	.02000	.23850	.934	4811	.5211
	9	.43000	.23850	.088	0711	.9311
	1	13000	.23850	.592	6311	.3711
	2	.11000	.23850	.650	3911	.6111
	3	.22000	.23850	.369	2811	.7211
л	5	.47000	.23850	.064	0311	.9711
4	6	.13000	.23850	.592	3711	.6311
	7	.14000	.23850	.564	3611	.6411
	8	.24000	.23850	.328	2611	.7411
	9	.65000*	.23850	.014	.1489	1.1511

	1	60000*	.23850	.022	-1.1011	0989
	2	36000	.23850	.149	8611	.1411
	3	25000	.23850	.308	7511	.2511
F	4	47000	.23850	.064	9711	.0311
5	6	34000	.23850	.171	8411	.1611
	7	33000	.23850	.183	8311	.1711
	8	23000	.23850	.348	7311	.2711
	9	.18000	.23850	.460	3211	.6811
	1	26000	.23850	.290	7611	.2411
	2	02000	.23850	.934	5211	.4811
	3	.09000	.23850	.710	4111	.5911
6	4	13000	.23850	.592	6311	.3711
0	5	.34000	.23850	.171	1611	.8411
	7	.01000	.23850	.967	4911	.5111
	8	.11000	.23850	.650	3911	.6111
	9	.52000*	.23850	.043	.0189	1.0211
	1	27000	.23850	.272	7711	.2311
	2	03000	.23850	.901	5311	.4711
	3	.08000	.23850	.741	4211	.5811
7	4	14000	.23850	.564	6411	.3611
'	5	.33000	.23850	.183	1711	.8311
	6	01000	.23850	.967	5111	.4911
	8	.10000	.23850	.680	4011	.6011
	9	.51000*	.23850	.046	.0089	1.0111
	1	37000	.23850	.138	8711	.1311
	2	13000	.23850	.592	6311	.3711
	3	02000	.23850	.934	5211	.4811
8	4	24000	.23850	.328	7411	.2611
0	5	.23000	.23850	.348	2711	.7311
	6	11000	.23850	.650	6111	.3911
	7	10000	.23850	.680	6011	.4011
	9	.41000	.23850	.103	0911	.9111
	1	78000*	.23850	.004	-1.2811	2789
	2	54000*	.23850	.036	-1.0411	0389
	3	43000	.23850	.088	9311	.0711
	4	65000*	.23850	.014	-1.1511	1489
9	5	18000	.23850	.460	6811	.3211
	6	52000 [*]	.23850	.043	-1.0211	0189
	7	51000*	.23850	.046	-1.0111	0089
	8	41000	.23850	.103	9111	.0911

E.2.5 Statistical analysis of orthogonal test for bacteria growth

Dependent Variable: Visible cell counts /log CFU /mL							
Source	Type III Sum of Squares	df	Mean Square	F	Sig.		
Corrected Model	.370ª	6	.062	1.681	.419		
Intercept	642.116	1	642.116	17528.178	.000		
Fermentation temperature	.115	2	.057	1.568	.389		
Fermentation time	.185	2	.093	2.530	.283		
Protein concentration	.069	2	.035	.945	.514		
Error	.073	2	.037				
Total	642.558	9					
Corrected Total	.443	8					

Tests of Between-Subjects Effects

a. R Squared = .835 (Adjusted R Squared = .338)



Estimated Marginal Means of Cell count / Log cfu/ml





E.2.6 Statistical analysis of sensory evaluation of semi-trained sensory panel (n=18) of the orthogonal test

	N Mean		Std.	Std. Error	95% Confiden Me	ce Interval for ean	Minimum	Maximum	
			Deviation		Lower Bound	Upper Bound			
1	3	6.5000	.50000	.28868	5.2579	7.7421	6.00	7.00	
2	3	5.5000	.50000	.28868	4.2579	6.7421	5.00	6.00	
3	3	4.6667	.15275	.08819	4.2872	5.0461	4.50	4.80	
4	3	5.6667	.15275	.08819	5.2872	6.0461	5.50	5.80	
5	3	4.0000	0.00000	0.00000	4.0000	4.0000	4.00	4.00	
6	3	6.2000	.50000	.28868	4.9579	7.4421	5.70	6.70	
7	3	6.1667	.35119	.20276	5.2943	7.0391	5.80	6.50	
8	3	5.8333	.35119	.20276	4.9609	6.7057	5.50	6.20	
9	3	4.5000	.20000	.11547	4.0032	4.9968	4.30	4.70	
Total	27	5.4481	.88028	.16941	5.0999	5.7964	4.00	7.00	

Descriptives

Average score of overall acceptability

Test of Homogeneity of Variances

Average score of overall acceptability

Levene Statistic	df1	df2	Sig.
1.163	8	18	.372

ANOVA

Average score of overall acceptability

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	17.981	8	2.248	18.672	.000
Within Groups	2.167	18	.120		
Total	20.147	26			

Multiple Comparisons

Dependent Variable: Average score of overall acceptability LSD

(l) Experimental number		Mean	Std. Error	0.	95% Confide	ence Interval
		Difference (I-J)	Std. Error	Sig.	Lower Bound	Upper Bound
	2	1.00000*	.28328	.002	.4049	1.5951
	3	1.83333*	.28328	.000	1.2382	2.4285
	4	.83333 [*]	.28328	.009	.2382	1.4285
1	5	2.50000*	.28328	.000	1.9049	3.0951
T	6	.30000	.28328	.304	2951	.8951
	7	.33333	.28328	.255	2618	.9285
	8	.66667*	.28328	.030	.0715	1.2618
	9	2.00000*	.28328	.000	1.4049	2.5951
2	1	-1.00000*	.28328	.002	-1.5951	4049

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	3	.83333 [*]	.28328	.009	.2382	1.4285
	4	16667	.28328	.564	7618	.4285
	5	1.50000*	.28328	.000	.9049	2.0951
	6	70000*	.28328	.024	-1.2951	1049
	7	66667*	.28328	.030	-1.2618	0715
	8	33333	.28328	.255	9285	.2618
	9	1.00000*	.28328	.002	.4049	1.5951
	1	-1.83333*	.28328	.000	-2.4285	-1.2382
	2	83333*	.28328	.009	-1.4285	2382
	4	-1.00000*	.28328	.002	-1.5951	4049
2	5	.66667*	.28328	.030	.0715	1.2618
3	6	-1.53333 [*]	.28328	.000	-2.1285	9382
	7	-1.50000 [*]	.28328	.000	-2.0951	9049
	8	-1.16667*	.28328	.001	-1.7618	5715
	9	.16667	.28328	.564	4285	.7618
	1	83333*	.28328	.009	-1.4285	2382
	2	.16667	.28328	.564	4285	.7618
	3	1.00000*	.28328	.002	.4049	1.5951
1	5	1.66667*	.28328	.000	1.0715	2.2618
4	6	53333	.28328	.076	-1.1285	.0618
	7	50000	.28328	.095	-1.0951	.0951
	8	16667	.28328	.564	7618	.4285
	9	1.16667^{*}	.28328	.001	.5715	1.7618
	1	-2.50000*	.28328	.000	-3.0951	-1.9049
	2	-1.50000°	.28328	.000	-2.0951	9049
	3	66667*	.28328	.030	-1.2618	0715
5	4	-1.66667*	.28328	.000	-2.2618	-1.0715
5	6	-2.20000°	.28328	.000	-2.7951	-1.6049
	7	-2.16667*	.28328	.000	-2.7618	-1.5715
	8	-1.83333*	.28328	.000	-2.4285	-1.2382
	9	50000	.28328	.095	-1.0951	.0951
	1	30000	.28328	.304	8951	.2951
	2	.70000*	.28328	.024	.1049	1.2951
	3	1.53333*	.28328	.000	.9382	2.1285
6	4	.53333	.28328	.076	0618	1.1285
-	5	2.20000*	.28328	.000	1.6049	2.7951
	7	.03333	.28328	.908	5618	.6285
	8	.36667	.28328	.212	2285	.9618
	9	1.70000*	.28328	.000	1.1049	2.2951
	1	33333	.28328	.255	9285	.2618
	2	.66667*	.28328	.030	.0715	1.2618
	3	1.50000*	.28328	.000	.9049	2.0951
7	4	.50000	.28328	.095	0951	1.0951
	5	2.16667*	.28328	.000	1.5715	2.7618
	6	03333	.28328	.908	6285	.5618
	8	.33333	.28328	.255	2618	.9285
	9	1.66667*	.28328	.000	1.0715	2.2618
8	1	66667*	.28328	.030	-1.2618	0715
	2	.33333	.28328	.255	2618	.9285

	3	1.16667^{*}	.28328	.001	.5715	1.7618
	4	.16667	.28328	.564	4285	.7618
	5	1.83333*	.28328	.000	1.2382	2.4285
	6	36667	.28328	.212	9618	.2285
	7	33333	.28328	.255	9285	.2618
	9	1.33333*	.28328	.000	.7382	1.9285
	1	-2.00000*	.28328	.000	-2.5951	-1.4049
	2	-1.00000*	.28328	.002	-1.5951	4049
	3	16667	.28328	.564	7618	.4285
0	4	-1.16667*	.28328	.001	-1.7618	5715
9	5	.50000	.28328	.095	0951	1.0951
	6	-1.70000*	.28328	.000	-2.2951	-1.1049
	7	-1.66667*	.28328	.000	-2.2618	-1.0715
	8	-1.33333*	.28328	.000	-1.9285	7382

E.2.7 Statistical analysis of orthogonal test for semi-trained sensory panel (n=18) sensory evaluation

De	Dependent Variable: Semi-trained sensory panel sensory score										
Source	Type III Sum of Squares	df	Mean Square	F	Sig.						
Corrected Model	4.527ª	6	.754	1.147	.535						
Intercept	266.778	1	266.778	405.574	.002						
Fermentation temperature	.149	2	.074	.113	.898						
Fermentation time	2.136	2	1.068	1.623	.381						
Protein concentration	2.242	2	1.121	1.704	.370						
Error	1.316	2	.658								
Total	272.620	9									
Corrected Total	5.842	8									

Tests of Between-Subjects Effects

a. R Squared = .775 (Adjusted R Squared = .099)





E.2.8 Statistical analysis of consumer	sensory evaluation (n=90) of final fermented pea
protein-coconut milk beverage	

Descriptives											
		N	Mean	Std.	Std Error	95% Confiden Me	ce Interval for ean	Minimu	Maximu		
		Deviation		Sta. Enoi	Lower Bound	Upper Bound	m	m			
	1	90	6.6667	1.09133	.11504	6.4381	6.8952	5.00	8.00		
Appearance	6	90	6.3667	1.14607	.12081	6.1266	6.6067	4.00	8.00		
Appearance	7	90	6.4000	1.17846	.12422	6.1532	6.6468	4.00	8.00		
	Total	270	6.4778	1.14289	.06955	6.3408	6.6147	4.00	8.00		
	1	90	6.7000	1.13623	.11977	6.4620	6.9380	4.00	8.00		
Aroma	6	90	6.6000	1.23434	.13011	6.3415	6.8585	5.00	9.00		
Aroma	7	90	6.4000	1.14950	.12117	6.1592	6.6408	5.00	8.00		
	Total	270	6.5667	1.17644	.07160	6.4257	6.7076	4.00	9.00		
	1	90	5.7000	1.25823	.13263	5.4365	5.9635	2.00	8.00		
Sourness	6	90	4.7333	1.67466	.17652	4.3826	5.0841	2.00	8.00		
300111833	7	90	5.1000	1.35787	.14313	4.8156	5.3844	3.00	8.00		
	Total	270	5.1778	1.49032	.09070	4.9992	5.3563	2.00	8.00		
	1	90	5.3222	1.47509	.15549	5.0133	5.6312	2.00	8.00		
Sweetness	6	90	5.2000	1.58788	.16738	4.8674	5.5326	2.00	9.00		
Sweetness	7	90	5.5000	1.15389	.12163	5.2583	5.7417	3.00	8.00		
	Total	270	5.3407	1.41769	.08628	5.1709	5.5106	2.00	9.00		
	1	90	5.7667	1.41461	.14911	5.4704	6.0630	2.00	8.00		
Flavour	6	90	5.3000	1.80168	.18991	4.9226	5.6774	1.00	9.00		
	7	90	5.3333	1.62840	.17165	4.9923	5.6744	2.00	8.00		

	Total	270	5.4667	1.63056	.09923	5.2713	5.6620	1.00	9.00
	1	90	6.1000	1.26358	.13319	5.8353	6.3647	3.00	9.00
Mouth fool	6	90	5.5000	1.83137	.19304	5.1164	5.8836	2.00	9.00
Modelliteel	7	90	5.1333	1.77498	.18710	4.7616	5.5051	1.00	9.00
	Total	270	5.5778	1.68513	.10255	5.3759	5.7797	1.00	9.00
	1	90	5.9667	1.47983	.15599	5.6567	6.2766	2.00	9.00
After teste	6	90	5.4667	1.43941	.15173	5.1652	5.7681	2.00	9.00
Aller laste	7	90	5.3000	1.44914	.15275	4.9965	5.6035	2.00	8.00
	Total	270	5.5778	1.47830	.08997	5.4006	5.7549	2.00	9.00
	1	90	6.2000	1.41580	.14924	5.9035	6.4965	2.00	8.00
Overall	6	90	5.4833	1.74570	.18401	5.1177	5.8490	2.00	9.00
acceptability	7	90	5.4667	1.36736	.14413	5.1803	5.7531	2.00	8.00
	Total	270	5.7167	1.55156	.09443	5.5308	5.9026	2.00	9.00

ANOVA									
		Sum of Squares	df	Mean Square	F	Sig.			
	Between Groups	4.867	2	2.433	1.875	.155			
Appearance	Within Groups	346.500	267	1.298					
	Total	351.367	269						
	Between Groups	4.200	2	2.100	1.523	.220			
Aroma	Within Groups	368.100	267	1.379					
	Total	372.300	269						
	Between Groups	42.867	2	21.433	10.319	.000			
Sourness	Within Groups	554.600	267	2.077					
	Total	597.467	269						
Sweetness	Between Groups	4.096	2	2.048	1.019	.362			
	Within Groups	536.556	267	2.010					
	Total	540.652	269						
	Between Groups	12.200	2	6.100	2.317	.101			
Flavour	Within Groups	703.000	267	2.633					
	Total	715.200	269						
	Between Groups	42.867	2	21.433	7.937	.000			
Mouth feel	Within Groups	721.000	267	2.700					
	Total	763.867	269						
	Between Groups	21.667	2	10.833	5.109	.007			
After taste	Within Groups	566.200	267	2.121					
	Total	587.867	269						
	Between Groups	31.550	2	15.775	6.837	.001			
Overall acceptability	Within Groups	616.025	267	2.307					
	Total	647.575	269						

Multiple Comparisons

Test of Homogeneity of Variances										
	Levene Statistic	df1	df2	Sig.						
Appearance	.308	2	267	.735						
Aroma	1.502	2	267	.224						
Sourness	8.349	2	267	.000						
Sweetness	2.536	2	267	.081						
Flavour	3.971	2	267	.020						
Mouth feel	10.171	2	267	.000						
After taste	.065	2	267	.937						
Overall acceptability	2.826	2	267	.061						
	LSD									

Mean 95% Confidence Interval Dependent Variable: Sample Difference (I-Std. Error Sig. Upper number Lower Bound J) Bound 6 .30000 .16982 .078 -.0344 .6344 1 7 .26667 .16982 .118 -.0677 .6010 .16982 -.6344 1 -.30000 .078 .0344 6 Appearance 7 -.03333 .16982 .845 -.3677 .3010 1 -.26667 .16982 .118 -.6010 .0677 7 6 -.3010 .03333 .16982 .845 .3677 6 .10000 .17503 .568 -.2446 .4446 1 7 .30000 .17503 .088 -.0446 .6446 1 -.10000 .17503 .568 -.4446 .2446 Aroma 6 7 .20000 .17503 .254 -.1446 .5446 1 -.30000 .17503 .088 -.6446 .0446 7 6 -.20000 .17503 .254 -.5446 .1446 6 .96667* .21485 .000 .5437 1.3897 1 7 .60000* .21485 .006 .1770 1.0230 .21485 -1.3897 -.96667* .000 -.5437 1 Sourness 6 7 -.36667 .21485 .089 -.7897 .0563 -.60000* .21485 .006 -1.0230 1 -.1770 7 6 .36667 .21485 .089 -.0563 .7897 6 .12222 .21132 .564 -.2938 .5383 1 7 -.17778 .21132 .401 -.5938 .2383 1 -.12222 .21132 .564 -.5383 .2938 6 Sweetness 7 -.30000 .21132 .157 -.7161 .1161 1 .17778 .21132 .401 -.2383 .5938 7 6 -.1161 .30000 .21132 .157 .7161 6 .46667 .24189 .055 -.0096 .9429 1 7 .43333 .24189 .074 -.0429 .9096 1 -.46667 .24189 .055 -.9429 .0096 Flavour 6 7 -.03333 .24189 .890 -.5096 .4429 -.43333 .24189 .074 -.9096 .0429 1 7 6 .03333 .24189 .890 -.4429 .5096 6 .60000* .24497 .015 .1177 1.0823 1 7 .24497 .000 .4844 1.4490 .96667* 1 -.60000* .24497 .015 -1.0823 -.1177 Mouth feel 6 7 .36667 .24497 .136 -.1156 .8490 -.4844 1 -.96667* .24497 .000 -1.4490 7 6 -.36667 .24497 .136 -.8490 .1156 6 .50000* .21708 .0726 .022 .9274 1 7 .66667* .21708 .002 .2393 1.0941 -.9274 1 -.50000* .21708 .022 -.0726 After taste 6 7 .16667 .21708 .443 -.2607 .5941 1 -.66667 .21708 .002 -1.0941 -.2393 7 6 -.16667 .21708 .443 -.5941 .2607 1 .2708 Overall acceptability 6 .71667* .22643 .002 1.1625

1 71667 ⁺ .22643 .002 -1.1625 2708 7 .01667 .22643 .941 4292 .4625 7 1 73333 ⁺ .22643 .001 -1.1792 2875 6 01667 .22643 .941 4625 .4292		7	.73333*	.22643	.001	.2875	1.1792
7 .01667 .22643 .941 4292 .4625 1 73333 [*] .22643 .001 -1.1792 2875 6 01667 .22643 .941 4625 .4292	6	1	71667*	.22643	.002	-1.1625	2708
1 73333' .22643 .001 -1.1792 2875 7 6 01667 .22643 .941 4625 .4292	0	7	.01667	.22643	.941	4292	.4625
⁷ 601667 .22643 .9414625 .4292	7	1	73333*	.22643	.001	-1.1792	2875
	1	6	01667	.22643	.941	4625	.4292

E.2.9 Statistical analysis of pH of fermented pea protein-coconut milk beverage during the fermentation Descriptives

				рн					
Time = (h)	N	Maan	Std.	Std.	95% Cor Interval f	nfidence for Mean	Miningung	Maximum	
nme (n)	IN	wean	Deviation	Error	Lower Bound	Upper Bound	Minimum	WIDXIIIIUIII	
0	3	6.1533	.12741	.07356	5.8368	6.4698	6.07	6.30	
2	3	5.9867	.06028	.03480	5.8369	6.1364	5.93	6.05	
4	3	5.1367	.09452	.05457	4.9019	5.3715	5.03	5.21	
6	3	4.5400	.04583	.02646	4.4262	4.6538	4.50	4.59	
8	3	4.2900	.01732	.01000	4.2470	4.3330	4.28	4.31	
Total	15	5.2213	.77652	.20050	4.7913	5.6514	4.28	6.30	

рН

Multiple Comparisons Dependent Variable: pH

			LJ			
(I) Time (h)		Mean			95% Confide	ence Interval
(I) -	Fime (h)	Difference (I-J)	Std. Error	Sig.	Lower Bound	Upper Bound
	2	.16667*	.06450	.027	.0230	.3104
0	4	1.01667*	.06450	.000	.8730	1.1604
0	6	1.61333*	.06450	.000	1.4696	1.7570
	8	1.86333*	.06450	.000	1.7196	2.0070
	0	16667*	.06450	.027	3104	0230
2	4	.85000*	.06450	.000	.7063	.9937
2	6	1.44667*	.06450	.000	1.3030	1.5904
	8	1.69667*	.06450	.000	1.5530	1.8404
	0	-1.01667*	.06450	.000	-1.1604	8730
4	2	85000*	.06450	.000	9937	7063
4	6	.59667*	.06450	.000	.4530	.7404
	8	.84667*	.06450	.000	.7030	.9904
	0	-1.61333*	.06450	.000	-1.7570	-1.4696
6	2	-1.44667*	.06450	.000	-1.5904	-1.3030
0	4	59667*	.06450	.000	7404	4530
	8	.25000*	.06450	.003	.1063	.3937
	0	-1.86333*	.06450	.000	-2.0070	-1.7196
0	2	-1.69667*	.06450	.000	-1.8404	-1.5530
0	4	84667*	.06450	.000	9904	7030
	6	25000*	.06450	.003	3937	1063

Test of Homogeneity of Variances

рН						
Levene Statistic	df1	df2	Sig.			
3.535	4	10	.048			

ANOVA	
nН	

	μn							
	Sum of Squares	df	Mean Square	F	Sig.			
Between Groups	8.379	4	2.095	335.712	.000			
Within Groups	.062	10	.006					
Total	8.442	14						

E.2.10 Statistical analysis of titratable acid of fermented pea protein-coconut milk beverage during the fermentation

Descriptives

T.A. (%)

Time (b)				Std.	95% Confidence Interval for Mean		Miningung	
nime (n)	IN	Mean	Deviation	Error	Lower Bound	Upper Bound	winimum	Maximum
0	3	.0933	.00577	.00333	.0790	.1077	.09	.10
2	3	.1200	.01000	.00577	.0952	.1448	.11	.13
4	3	.2467	.02517	.01453	.1842	.3092	.22	.27
6	3	.3367	.02517	.01453	.2742	.3992	.31	.36
8	3	.5167	.02517	.01453	.4542	.5792	.49	.54
Total	15	.2627	.16078	.04151	.1736	.3517	.09	.54

Test of Homogeneity of Variances

T.A. (%)

Levene Statistic	df1	df2	Sig.
1.324	4	10	.326

_	T.A. (%)								
	Sum of Squares	df	Mean Square	F	Sig.				
Between Groups	.358	4	.089	219.975	.000				
Within Groups	.004	10	.000						
Total	.362	14							

Multiple Comparisons

	LSD							
		Mean			95% Confidence Interval			
(I)	Time (h)	Difference (I-J)	Std. Error	Sig.	Lower Bound	Upper Bound		
	2	02667	.01647	.136	0634	.0100		
0	4	15333 [*]	.01647	.000	1900	1166		
0	6	24333 [*]	.01647	.000	2800	2066		
	8	42333 [*]	.01647	.000	4600	3866		
	0	.02667	.01647	.136	0100	.0634		
2	4	12667*	.01647	.000	1634	0900		
2	6	21667*	.01647	.000	2534	1800		
	8	39667*	.01647	.000	4334	3600		
	0	.15333*	.01647	.000	.1166	.1900		
4	2	.12667*	.01647	.000	.0900	.1634		
4	6	09000*	.01647	.000	1267	0533		
	8	27000 [*]	.01647	.000	3067	2333		
	0	.24333*	.01647	.000	.2066	.2800		
6	2	.21667*	.01647	.000	.1800	.2534		
0	4	.09000*	.01647	.000	.0533	.1267		
	8	18000*	.01647	.000	2167	1433		
	0	.42333*	.01647	.000	.3866	.4600		
0	2	.39667*	.01647	.000	.3600	.4334		
0	4	.27000*	.01647	.000	.2333	.3067		
	6	.18000*	.01647	.000	.1433	.2167		

Dependent Variable: T.A. (%)

 $\star.$ The mean difference is significant at the 0.05 level.

E.2.11 Statistical analysis of VCCs (log CFU /mL) of fermented pea protein-coconut milk beverage during the fermentation

Bacterial quantity (log CFU /mL)									
	N		Std.	95% Con Std. Interval fe		95% Confidence Interval for Mean		M	
nme (n)	N	wean	Deviation	Error	Lower Bound	Upper Bound	winimum	Waximum	
0	3	5.5400	.10000	.05774	5.2916	5.7884	5.44	5.64	
2	3	6.9900	.28000	.16166	6.2944	7.6856	6.71	7.27	
4	3	7.8500	.30000	.17321	7.1048	8.5952	7.55	8.15	
6	3	8.1800	.34000	.19630	7.3354	9.0246	7.84	8.52	
8	3	8.6100	.35000	.20207	7.7406	9.4794	8.26	8.96	
Total	15	7.4340	1.15027	.29700	6.7970	8.0710	5.44	8.96	

Descriptives

Test of Homogeneity of Variances

Bacterial quantity (log CFU /mL)

Levene Statistic	df1	df2	Sig.
.494	4	10	.741

ANOVA

Bacterial quantity (log CFU /mL)

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	17.691	4	4.423	53.094	.000
Within Groups	.833	10	.083		
Total	18.524	14			

Multiple Comparisons

Dependent Variable: Bacterial quantity (log CFU /mL)

				LSD				
		Mean			95% Confide	95% Confidence Interval		
(I) Time (h)		Difference (I-J)	Std. Error	Sig.	Lower Bound	Upper Bound		
	2	-1.45000*	.23566	.000	-1.9751	9249		
0	4	-2.31000*	.23566	.000	-2.8351	-1.7849		
0	6	-2.64000*	.23566	.000	-3.1651	-2.1149		
	8	-3.07000*	.23566	.000	-3.5951	-2.5449		
	0	1.45000*	.23566	.000	.9249	1.9751		
2	4	86000*	.23566	.004	-1.3851	3349		
2	6	-1.19000*	.23566	.000	-1.7151	6649		
	8	-1.62000*	.23566	.000	-2.1451	-1.0949		
	0	2.31000*	.23566	.000	1.7849	2.8351		
Л	2	.86000*	.23566	.004	.3349	1.3851		
4	6	33000	.23566	.192	8551	.1951		
	8	76000*	.23566	.009	-1.2851	2349		
	0	2.64000*	.23566	.000	2.1149	3.1651		
6	2	1.19000*	.23566	.000	.6649	1.7151		
0	4	.33000	.23566	.192	1951	.8551		
	8	43000	.23566	.098	9551	.0951		
	0	3.07000*	.23566	.000	2.5449	3.5951		
8	2	1.62000*	.23566	.000	1.0949	2.1451		
0	4	.76000*	.23566	.009	.2349	1.2851		
6	.43000	.23566	.098	0951	.9551			

E.2.12 Statistical analysis of colour of fermented pea protein-coconut milk beverage during the fermentation

	Descriptives									
		N	Maaa	Std.	Std.	95% Cor Interval f	nfidence for Mean	N disainan una	Maria	
Time (h)		IN	Mean	Deviation	Error	Lower Bound	Upper Bound	winimum	Waximam	
	0	3	73.3967	.27154	.15677	72.7221	74.0712	73.23	73.71	
	2	3	73.5967	.14572	.08413	73.2347	73.9586	73.46	73.75	
1.*	4	3	74.2133	.38371	.22154	73.2601	75.1665	73.93	74.65	
L	6	3	74.2667	.07506	.04333	74.0802	74.4531	74.19	74.34	
	8	3	74.4767	.25502	.14723	73.8432	75.1102	74.22	74.73	
	Total	15	73.9900	.48076	.12413	73.7238	74.2562	73.23	74.73	
	0	3	.0333	.00577	.00333	.0190	.0477	.03	.04	
	2	3	.0267	.00577	.00333	.0123	.0410	.02	.03	
. *	4	3	.0133	.00577	.00333	0010	.0277	.01	.02	
a	6	3	0167	.00577	.00333	0310	0023	02	01	
	8	3	0233	.00577	.00333	0377	0090	03	02	
	Total	15	.0067	.02410	.00622	0067	.0200	03	.04	
	0	3	2.9933	.04726	.02728	2.8759	3.1107	2.94	3.03	
	2	3	3.5667	.41501	.23961	2.5357	4.5976	3.15	3.98	
b*	4	3	4.1367	.48003	.27715	2.9442	5.3291	3.77	4.68	
U	6	3	4.8767	.25541	.14746	4.2422	5.5111	4.59	5.08	
	8	3	4.9567	.30827	.17798	4.1909	5.7225	4.61	5.20	
	Total	15	4.1060	.83120	.21462	3.6457	4.5663	2.94	5.20	

			ANOVA			
		Sum of Squares	df	Mean Square	F	Sig.
	Between Groups	2.610	4	.653	10.428	.001
L	Within Groups	.626	10	.063		
	Total	3.236	14			
	Between Groups	.008	4	.002	58.500	.000
a [*]	Within Groups	.000	10	.000		
	Total	.008	14			
	Between Groups	8.542	4	2.136	18.893	.000
b,	Within Groups	1.130	10	.113		
	Total	9.673	14			

Test of Homogeneity of Variances

	Levene Statistic	df1	df2	Sig.
L*	2.297	4	10	.131
a*	.000	4	10	1.000
b	1.895	4	10	.188

Multiple Comparisons

				LSD			
Descendent Ve	al a la l		Mean			95% Cor	nfidence
Dependent Va Time (h)	iriabi 1	e:	Difference	Std. Error	Sig.	Lower	
			(I-J)			Bound	Bound
		2	20000	.20424	.351	6551	.2551
	0	4	81667*	.20424	.003	-1.2718	3616
	0	6	87000*	.20424	.002	-1.3251	4149
		8	-1.08000*	.20424	.000	-1.5351	6249
		0	.20000	.20424	.351	2551	.6551
	2	4	61667*	.20424	.013	-1.0718	1616
	Ζ	6	67000*	.20424	.008	-1.1251	2149
		8	88000*	.20424	.002	-1.3351	4249
		0	.81667*	.20424	.003	.3616	1.2718
1*	4	2	.61667*	.20424	.013	.1616	1.0718
L	4	6	05333	.20424	.799	5084	.4018
		8	26333	.20424	.226	7184	.1918
		0	.87000*	.20424	.002	.4149	1.3251
	6	2	.67000*	.20424	.008	.2149	1.1251
	0	4	.05333	.20424	.799	4018	.5084
		8	21000	.20424	.328	6651	.2451
	8	0	1.08000*	.20424	.000	.6249	1.5351
		2	.88000*	.20424	.002	.4249	1.3351
		4	.26333	.20424	.226	1918	.7184
		6	.21000	.20424	.328	2451	.6651
		2	.00667	.00471	.188	0038	.0172
	0	4	.02000*	.00471	.002	.0095	.0305
		6	.05000*	.00471	.000	.0395	.0605
		8	.05667*	.00471	.000	.0462	.0672
		0	00667	.00471	.188	0172	.0038
	2	4	.01333*	.00471	.018	.0028	.0238
		6	.04333*	.00471	.000	.0328	.0538
		8	.05000*	.00471	.000	.0395	.0605
		0	02000*	.00471	.002	0305	0095
a*	4	2	01333*	.00471	.018	0238	0028
		6	.03000*	.00471	.000	.0195	.0405
		8	.03667	.00471	.000	.0262	.0472
		0	05000	.00471	.000	0605	0395
	6	2	04333	.00471	.000	0538	0328
		4	03000	.00471	.000	0405	0195
		8	.00667	.00471	.188	0038	.0172
		0	U5667	.00471	.000	0672	0462
	8	2	U5UUU	.00471	.000	0605	0395
		4	U3007	.00471	.000	0472	U262
		ю Э	00007	.00471 27451	.188	U1/2	.0038
	~	۷	5/333	.27451	.003	-1.1820	.0383
d	U	4	-1.14333	.27451	.002	-1./550	5317
	_	6	-1.88333*	.27451	.000	-2.4950	-1.2717

	8	-1.96333*	.27451	.000	-2.5750	-1.3517
	0	.57333	.27451	.063	0383	1.1850
2	4	57000	.27451	.065	-1.1816	.0416
Z	6	-1.31000*	.27451	.001	-1.9216	6984
	8	-1.39000*	.27451	.000	-2.0016	7784
	0	1.14333*	.27451	.002	.5317	1.7550
4	2	.57000	.27451	.065	0416	1.1816
4	6	74000*	.27451	.022	-1.3516	1284
	8	82000 [*]	.27451	.014	-1.4316	2084
	0	1.88333*	.27451	.000	1.2717	2.4950
6	2	1.31000*	.27451	.001	.6984	1.9216
0	4	.74000*	.27451	.022	.1284	1.3516
	8	08000	.27451	.777	6916	.5316
	0	1.96333*	.27451	.000	1.3517	2.5750
0	2	1.39000*	.27451	.000	.7784	2.0016
8	4	.82000*	.27451	.014	.2084	1.4316
	6	.08000	.27451	.777	5316	.6916

E.2.13 Statistical analysis of sugars and organic acids in fermented pea protein-coconut milk beverage during fermentation

Descriptives									
						95% Cor	nfidence		
		N	Maan	Std.	Std.	Interval for Mean		Minimum	Maximum
Fermentation	time (h)	IN	IVIEdIT	Deviation	Error	Lower	Upper	wiminium	IVIAXITTUTT
						Bound	Bound		
	0	3	2.2033	.05774	.03333	2.0599	2.3468	2.17	2.27
Sucroso	2	3	2.0567	.05774	.03333	1.9132	2.2001	1.99	2.09
sucrose	4	3	2.0433	.05774	.03333	1.8999	2.1868	2.01	2.11
(% w (v)	6	3	1.9333	.05774	.03333	1.7899	2.0768	1.90	2.00
(% VV / V)	8	3	1.8333	.04163	.02404	1.7299	1.9368	1.80	1.88
	Total	15	2.0140	.13710	.03540	1.9381	2.0899	1.80	2.27
	0	3	.1433	.00577	.00333	.1290	.1577	.14	.15
Fructoro	2	3	.1700	.01000	.00577	.1452	.1948	.16	.18
concontration	4	3	.2133	.01528	.00882	.1754	.2513	.20	.23
(% w (v)	6	3	.2433	.02082	.01202	.1916	.2950	.22	.26
(70 VV / V)	8	3	.2667	.02082	.01202	.2150	.3184	.25	.29
	Total	15	.2073	.04891	.01263	.1802	.2344	.14	.29
	0	3	.2367	.00577	.00333	.2223	.2510	.23	.24
Lactic acid	2	3	.3467	.01155	.00667	.3180	.3754	.34	.36
concentration	4	3	.6833	.06110	.03528	.5316	.8351	.63	.75
(% w (v)	6	3	.9467	.03512	.02028	.8594	1.0339	.91	.98
(/0 00 / 0)	8	3	1.2300	.09000	.05196	1.0064	1.4536	1.14	1.32
	Total	15	.6887	.38445	.09926	.4758	.9016	.23	1.32
	0	3	1.7300	.15395	.08888	1.3476	2.1124	1.56	1.86
	2	3	1.8767	.08505	.04910	1.6654	2.0879	1.78	1.94
Acetic acid	4	3	2.7067	.11930	.06888	2.4103	3.0030	2.61	2.84
concentration	6	3	3.6400	.15395	.08888	3.2576	4.0224	3.51	3.81
(% w /v)	8	3	4.0100	.17349	.10017	3.5790	4.4410	3.82	4.16
	Total	15	2.7927	.95337	.24616	2.2647	3.3206	1.56	4.16

Test of Homogeneity of Variances									
	Levene Statistic	df1	df2	Sig.					
Sucrose concentration (% w /v)	.303	4	10	.870					
Fructose concentration (% w /v)	1.705	4	10	.225					
Lactic acid concentration (% w /v)	2.182	4	10	.145					
Acetic acid concentration (% w /v)	.502	4	10	.736					

ANOVA	
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		Sum of Squares	df	Mean Square	F	Sig.
Sucrose	Between Groups	.233	4	.058	19.333	.000
concentration (% w /v)	Within Groups	.030	10	.003		
	Total	.263	14			
Fructose	Between Groups	.031	4	.008	31.446	.000
concentration (% w /v)	Within Groups	.002	10	.000		
	Total	.033	14			
Lactic acid	Between Groups	2.043	4	.511	192.951	.000
concentration (% w /v)	Within Groups	.026	10	.003		
	Total	2.069	14			
Acetic acid	Between Groups	12.527	4	3.132	158.219	.000
concentration (% w /v)	Within Groups	.198	10	.020		
	Total	12.725	14			

Multiple	Comparisons

-				LJD			
						95% Cor	nfidence
Dependent Vari	iable	:	Mean			Inte	erval
Fermentation tin	ne (h	1)	Difference	Std. Error	Sig.	Lower	Unner
i ennentation ai			(I-J)			Round	Round
		-	4 4 6 6 7 *			Bourio	Bound
		2	.14667	.04482	.008	.0468	.2465
	0	4	.16000*	.04482	.005	.0601	.2599
	0	6	.27000*	.04482	.000	.1701	.3699
		8	.37000*	.04482	.000	.2701	.4699
		0	14667*	.04482	.008	2465	0468
	_	4	.01333	.04482	.772	0865	.1132
	2	6	12333*	04482	020	0235	2232
		8	22333*	04482	001	1235	3232
		0	16000*	04492	005	2500	.0202
Sucrose		2	10000	.04402	.003	2.399	0001
concentration (%	4	2	01333	.04482	.112	1132	.0805
w /v)		6	.11000	.04482	.034	.0101	.2099
,		8	.21000	.04482	.001	.1101	.3099
		0	27000*	.04482	.000	3699	1701
	6	2	12333 [*]	.04482	.020	2232	0235
	0	4	11000 [*]	.04482	.034	2099	0101
		8	.10000*	.04482	.050	.0001	.1999
		0	37000*	.04482	.000	4699	2701
		2	22333*	.04482	.001	3232	1235
	8	4	- 21000*	04482	001	- 3099	- 1101
		6	10000*	04482	050	1000	.1101
		2	10000	.04402	.050	1999	0001
		2	02007	.01202	.004	0552	.0019
	0	4	07000	.01282	.000	0986	0414
		6	10000	.01282	.000	1286	0714
		8	12333	.01282	.000	1519	0948
		0	.02667	.01282	.064	0019	.0552
	2	4	04333*	.01282	.007	0719	0148
	2	6	07333*	.01282	.000	1019	0448
		8	09667*	.01282	.000	1252	0681
	4	0	.07000*	.01282	.000	.0414	.0986
Fructose		2	.04333*	.01282	.007	.0148	.0719
concentration (%		6	- 0.3000*	01282	041	- 0586	- 0014
w /v)		8	- 05333*	01282	002	- 0819	- 0248
		0	10000*	.01202	.002	0013	1296
		0	.10000	.01202	.000	.0714	.1200
		2	.07333	.01282	.000	.0448	.1019
		4	.03000	.01282	.041	.0014	.0586
		8	02333	.01282	.099	0519	.0052
		0	.12333*	.01282	.000	.0948	.1519
	Q	2	.09667*	.01282	.000	.0681	.1252
	0	4	.05333*	.01282	.002	.0248	.0819
		6	.02333	.01282	.099	0052	.0519
		2	11000*	.04201	.026	2036	0164
	~	4	44667*	.04201	.000	5403	3531
	U	6	71000*	.04201	.000	8036	6164
		8	- 99333*	.04201	.000	-1.0869	- 8997
		0	11000*	04201	026	0164	2036
		1	_ 33667*	0/201	000	- 1202	_ 2/21
	2	4 6	33007	04201	.000	4000	24JL
Lactic acid		0 C	00000	.04201	.000	0930	5004
concentration (%		8	88333	.04201	.000	9/69	/89/
w /v)		0	.44667	.04201	.000	.3531	.5403
,	Δ	2	.33667*	.04201	.000	.2431	.4303
	7	6	26333*	.04201	.000	3569	1697
		8	54667*	.04201	.000	6403	4531
		0	.71000*	.04201	.000	.6164	.8036
	~	2	.60000*	.04201	.000	.5064	.6936
	6	4	.26333*	.04201	.000	.1697	.3569
		8	28333*	.04201	.000	- 3769	- 1897
1		5					001

		0	.99333*	.04201	.000	.8997	1.0869
	Q	2	.88333*	.04201	.000	.7897	.9769
	0	4	.54667*	.04201	.000	.4531	.6403
		6	.28333*	.04201	.000	.1897	.3769
		2	14667	.11487	.231	4026	.1093
	0	4	97667*	.11487	.000	-1.2326	7207
	0	6	-1.91000*	.11487	.000	-2.1660	-1.6540
		8	-2.28000*	.11487	.000	-2.5360	-2.0240
		0	.14667	.11487	.231	1093	.4026
	2	4	83000*	.11487	.000	-1.0860	5740
	2	6	-1.76333*	.11487	.000	-2.0193	-1.5074
		8	-2.13333*	.11487	.000	-2.3893	-1.8774
	4	0	.97667*	.11487	.000	.7207	1.2326
Acetic acid		2	.83000*	.11487	.000	.5740	1.0860
w /v)	4	6	93333 [*]	.11487	.000	-1.1893	6774
****		8	-1.30333*	.11487	.000	-1.5593	-1.0474
		0	1.91000*	.11487	.000	1.6540	2.1660
	6	2	1.76333*	.11487	.000	1.5074	2.0193
	0	4	.93333*	.11487	.000	.6774	1.1893
		8	37000 [*]	.11487	.009	6260	1140
		0	2.28000*	.11487	.000	2.0240	2.5360
	0	2	2.13333*	.11487	.000	1.8774	2.3893
	0	4	1.30333*	.11487	.000	1.0474	1.5593
		6	.37000*	.11487	.009	.1140	.6260

E.2.14 Statistical analysis of protein content in fermented pea protein-coconut milk beverage during fermentation

Time (h)	N	Mean	Std. Std.		95% Confidence Interval for Mean		Minimum	Maximum
			Deviation	Error	Lower Bound	Upper Bound		
0	3	2.2200	.03000	.01732	2.1455	2.2945	2.19	2.25
2	3	2.2500	.06000	.03464	2.1010	2.3990	2.19	2.31
4	3	2.3133	.06506	.03756	2.1517	2.4750	2.25	2.38
6	3	2.3333	.04041	.02333	2.2329	2.4337	2.31	2.38
8	3	2.3767	.06506	.03756	2.2150	2.5383	2.31	2.44
Total	15	2.2987	.07434	.01919	2.2575	2.3398	2.19	2.44

Descriptives

Test of Homogeneity of Variances

Protein concentration (%)

Levene Statistic	df1	df2	Sig.
.389	4	10	.812

ANOVA

Protein concentration (%)

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.048	4	.012	4.124	.031
Within Groups	.029	10	.003		
Total	.077	14			

Multiple Comparisons

Dependent Variable: Protein concentration (%) LSD

				LOD		
(I)	Mean			95% Confide	ence Interval
Time (h)		Difference (I-J)	Std. Error	Sig.	Lower Bound	Upper Bound
	2	03000	.04412	.512	1283	.0683
0	4	09333	.04412	.060	1916	.0050
U	6	11333*	.04412	.028	2116	0150
	8	15667*	.04412	.005	2550	0584
	0	.03000	.04412	.512	0683	.1283
	4	06333	.04412	.182	1616	.0350
Ζ	6	08333	.04412	.088	1816	.0150
	8	12667*	.04412	.017	2250	0284
	0	.09333	.04412	.060	0050	.1916
	2	.06333	.04412	.182	0350	.1616
4	6	02000	.04412	.660	1183	.0783
	8	06333	.04412	.182	1616	.0350
	0	.11333*	.04412	.028	.0150	.2116
G	2	.08333	.04412	.088	0150	.1816
Ю	4	.02000	.04412	.660	0783	.1183
	8	04333	.04412	.349	1416	.0550
	0	.15667*	.04412	.005	.0584	.2550
0	2	.12667*	.04412	.017	.0284	.2250
8	4	.06333	.04412	.182	0350	.1616
	6	.04333	.04412	.349	0550	.1416

E.3 Statistical output phase 3: Stability of fermented fermented pea protein-coconut milk beverage during storage at 4 °C for 21 days

E.3.1 Statistical analysis of pH change in fermented pea protein-coconut milk beverage during storage (4 $^{\circ}$ C) for 21 days

-				рН				
			Std.	Std.	95% Cor Interval f	nfidence for Mean	Miningung	Maximum
	IN	wear	Deviation	Error	Lower	Upper	winimum	Waximum
					Bound	Bound		
1	3	4.4300	.03000	.01732	4.3555	4.5045	4.40	4.46
7	3	4.3900	.02000	.01155	4.3403	4.4397	4.37	4.41
14	3	4.3600	.04000	.02309	4.2606	4.4594	4.32	4.40
21	3	4.3800	.02000	.01155	4.3303	4.4297	4.36	4.40
Total	12	4.3900	.03618	.01044	4.3670	4.4130	4.32	4.46

Descriptives

Test of Homogeneity of Variances

	р	Н	
Levene Statistic	df1	df2	Sig.
.444	3	8	.728

ANOVA

-		р	Н		
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.008	3	.003	3.152	.086
Within Groups	.007	8	.001		
Total	.014	11			

Multiple Comparisons

Dependent Variable: pH

-				LOD		
((I) Mean				95% Confide	ence Interval
stor	rage	Difference	Std. Error	Sig.	Lower	Upper
da	ays	(I-J)			Bound	Bound
	7	.04000	.02345	.126	0141	.0941
1	14	.07000*	.02345	.017	.0159	.1241
	21	.05000	.02345	.066	0041	.1041
	1	04000	.02345	.126	0941	.0141
7	14	.03000	.02345	.237	0241	.0841
	21	.01000	.02345	.681	0441	.0641
	1	07000*	.02345	.017	1241	0159
14	7	03000	.02345	.237	0841	.0241
	21	02000	.02345	.419	0741	.0341
	1	05000	.02345	.066	1041	.0041
21	7	01000	.02345	.681	0641	.0441
	14	.02000	.02345	.419	0341	.0741

E.3.2 Statistical analysis of titratable acid change of fermented pea protein-coconut milk beverage during storage (4 $^{\circ}C$) for 21 days

	Descriptives								
				I.A. (%)					
					95% Cor	nfidence			
	N	Mean	Std.	Std.	Interval for Mean		Minimum	m Maximum	
		Ivican	Deviation	Error	Lower	Upper	Willinnun	Maximum	
					Bound	Bound			
1	3	.5300	.01000	.00577	.5052	.5548	.52	.54	
7	3	.5500	.01000	.00577	.5252	.5748	.54	.56	
14	3	.5600	.01000	.00577	.5352	.5848	.55	.57	
21	3	.5467	.00577	.00333	.5323	.5610	.54	.55	
Total	12	.5467	.01371	.00396	.5380	.5554	.52	.57	

Test of Homogeneity of Variances

	T.A	. (%)	
Levene Statistic	df1	df2	Sig.
.143	3	8	.931

	ANOVA T.A. (%)							
	Sum of Squares	df	Mean Square	F	Sig.			
Between Groups	.001	3	.000	5.600	.023			
Within Groups	.001	8	.000					
Total	.002	11						

Multiple Comparisons Dependent Variable: T.A. (%) LSD

([])	Mean			95% Confide	ence Interval
storage		Difference	Std. Error	Sig.	Lower	Upper
da	ays	(I-J)			Bound	Bound
	7	02000*	.00745	.028	0372	0028
1	14	03000*	.00745	.004	0472	0128
2	21	01667	.00745	.056	0339	.0005
	1	.02000*	.00745	.028	.0028	.0372
7	14	01000	.00745	.217	0272	.0072
	21	.00333	.00745	.667	0139	.0205
	1	.03000*	.00745	.004	.0128	.0472
14	7	.01000	.00745	.217	0072	.0272
	21	.01333	.00745	.111	0039	.0305
	1	.01667	.00745	.056	0005	.0339
21	7	00333	.00745	.667	0205	.0139
	14	01333	.00745	.111	0305	.0039

E.3.3 Statistical analysis of bacterial content of fermented pea protein-coconut milk beverage during storage (4 $^{\circ}$ C) for 21 days

_	Descriptives Bacteria quantity (Log CFU /mL)										
		N	.,	Std.	Std.	95% Cor Interval f	nfidence or Mean	Minimum	Maximum		
		IN.	Iviean	Deviation	Error	Lower Bound	Upper Bound	Willing	Waximum		
	1	3	8.6633	.03512	.02028	8.5761	8.7506	8.63	8.70		
	7	3	8.7067	.02082	.01202	8.6550	8.7584	8.69	8.73		
	14	3	8.7233	.02517	.01453	8.6608	8.7858	8.70	8.75		
	21	3	8.6967	.01528	.00882	8.6587	8.7346	8.68	8.71		
	Total	12	8.6975	.03137	.00906	8.6776	8.7174	8.63	8.75		

Test of Homogeneity of Variances

Bacteria quantity (Log CFU /mL)

Levene Statistic	df1	df2	Sig.	
.602	3	8	.631	

Г

ANOVA Bacteria quantity (Log CFU /mL) Sum of df Mean F Squares Square

	Squares	df	Square	F	Sig.
Between Groups	.006	3	.002	3.031	.093
Within Groups	.005	8	.001		
Total	.011	11			

Multiple Comparisons Dependent Variable: Bacteria quantity (Log CFU /mL) LSD

([])	Mean			95% Confide	ence Interval
Storage		Difference	Std. Error	Sig.	Lower	Upper
da	ays	(I-J)			Bound	Bound
	7	04333	.02055	.068	0907	.0041
1	14	06000*	.02055	.019	1074	0126
	21	03333	.02055	.143	0807	.0141
	1	.04333	.02055	.068	0041	.0907
7	14	01667	.02055	.441	0641	.0307
	21	.01000	.02055	.640	0374	.0574
	1	.06000*	.02055	.019	.0126	.1074
14	7	.01667	.02055	.441	0307	.0641
	21	.02667	.02055	.231	0207	.0741
	1	.03333	.02055	.143	0141	.0807
21	7	01000	.02055	.640	0574	.0374
	14	02667	.02055	.231	0741	.0207

E.3.4 Statistical analysis of colour of fermented pea protein-coconut milk beverage during storage (4 $^{\circ}$ C) for 21 days

	Descriptives										
С	Colour	N	M	Std.	Std.	95% Cor Interval f	nfidence for Mean		N4 -		
Sto	ore days	N	iviean	Deviation	Error	Lower	Upper	IVIINIMUM	Maximum		
						Bound	Bound				
	1	3	74.4767	.25502	.14723	73.8432	75.1102	74.22	74.73		
	7	3	75.3767	.61011	.35225	73.8611	76.8923	74.76	75.98		
L	14	3	75.7567	.16803	.09701	75.3393	76.1741	75.61	75.94		
	21	3	72.4067	1.37264	.79249	68.9968	75.8165	70.86	73.48		
	Total	12	74.5042	1.50424	.43424	73.5484	75.4599	70.86	75.98		
	1	3	0233	.00577	.00333	0377	0090	03	02		
	7	3	0167	.01155	.00667	0454	.0120	03	01		
а	14	3	.3133	.00577	.00333	.2990	.3277	.31	.32		
	21	3	.3767	.07095	.04096	.2004	.5529	.30	.44		
	Total	12	.1625	.19452	.05615	.0389	.2861	03	.44		
	1	3	4.9567	.30827	.17798	4.1909	5.7225	4.61	5.20		
	7	3	5.1700	.44034	.25423	4.0761	6.2639	4.84	5.67		
b	14	3	4.3433	.02887	.01667	4.2716	4.4150	4.31	4.36		
	21	3	4.1633	.22502	.12991	3.6044	4.7223	3.94	4.39		
	Total	12	4.6583	.50145	.14476	4.3397	4.9769	3.94	5.67		

	rest of fiomogeneity of variances										
	Levene Statistic	df1	df2	Sig.							
L	4.813	3	8	.034							
а	5.433	3	8	.025							
b	3.533	3	8	.068							

	ANOVA									
		Sum of Squares	df	Mean Square	F	Sig.				
L	Between Groups	20.191	3	6.730	11.458	.003				
	Within Groups	4.699	8	.587						
	Total	24.890	11							
	Between Groups	.406	3	.135	103.378	.000				
а	Within Groups	.010	8	.001						
	Total	.416	11							
b	Between Groups	2.085	3	.695	8.168	.008				
	Within Groups	.681	8	.085						
	Total	2.766	11							

Test of Homogeneity of Variances

				LJD			
			Mana			95% Cor	nfidence
Depender	nt Variab	le:	Difference	Std Error	Sig	Inte	erval
Store	e days			Stu. Enoi	siy.	Lower	Upper
			([-])			Bound	Bound
		7	90000	.62578	.188	-2.3431	.5431
	1	14	-1.28000	.62578	.075	-2.7231	.1631
		21	2.07000*	.62578	.011	.6269	3.5131
		0	.90000	.62578	.188	5431	2.3431
	7	14	38000	.62578	.561	-1.8231	1.0631
1		21	2.97000*	.62578	.001	1.5269	4.4131
L		0	1.28000	.62578	.075	1631	2.7231
	14	7	.38000	.62578	.561	-1.0631	1.8231
		21	3.35000*	.62578	.001	1.9069	4.7931
		0	-2.07000*	.62578	.011	-3.5131	6269
	21	7	-2.97000*	.62578	.001	-4.4131	-1.5269
		14	-3.35000*	.62578	.001	-4.7931	-1.9069
		7	00667	.02953	.827	0748	.0614
	1	14	33667	.02953	.000	4048	2686
		21	40000	.02953	.000	4681	3319
		0	.00667	.02953	.827	0614	.0748
	7	14	33000	.02953	.000	3981	2619
а		21	39333	.02953	.000	4614	3252
_		0	.33667	.02953	.000	.2686	.4048
	14	1	.33000	.02953	.000	.2619	.3981
		21	06333	.02953	.064	1314	.0048
		0	.40000	.02953	.000	.3319	.4681
	21		.39333	.02953	.000	.3252	.4614
		14	.06333	.02953	.064	0048	.1314
		(21333	.23819	.397	7626	.3359
	1	14	.61333*	.23819	.033	.0641	1.1626
		21	.79333*	.23819	.010	.2441	1.3426
		0	.21333	.23819	.397	3359	.7626
	7	14	.82667*	.23819	.008	.2774	1.3759
		21	1.00667*	.23819	.003	.4574	1.5559
b		0	61333*	.23819	.033	-1.1626	0641
	14	7	- 82667*	23819	008	-1 3759	- 2774
		21	18000	23819	471	- 3693	7293
		0	70332*	23810	010	1 3426	- 2441
	01	0 7	13000	.23013	.010	1 5550	2441 4F74
	21	1	-1.00007	.23819	.003	-1.5559	4574
1		14	- 18000	23819	471	- 7293	3693

Multiple Comparisons

E.3.5 Statistical analysis of sugar and organic acid change of fermented pea protein-coconut milk beverage during storage (4 $^{\circ}$ C) for 21 days

_	Descriptives											
		NI	Moon	Std.	Std.	95% Cor Interval f	nfidence for Mean	Minimum	Maximum			
Storage days		IN	IVIEdIT	Deviation	Error	Lower Bound	Upper Bound	wiiniiniuni	Waxinani			
	1	3	3.0467	.02517	.01453	2.9842	3.1092	3.02	3.07			
Sucrose	7	3	2.8000	.05000	.02887	2.6758	2.9242	2.75	2.85			
concentration	14	3	2.4200	.01000	.00577	2.3952	2.4448	2.41	2.43			
(% w /v)	21	3	1.6833	.00577	.00333	1.6690	1.6977	1.68	1.69			
	Total	12	2.4875	.53862	.15549	2.1453	2.8297	1.68	3.07			
	1	3	.1833	.02517	.01453	.1208	.2458	.16	.21			
Fructose	7	3	.2267	.03055	.01764	.1508	.3026	.20	.26			
concentration	14	3	.2467	.02517	.01453	.1842	.3092	.22	.27			
(% w /v)	21	3	.2667	.02517	.01453	.2042	.3292	.24	.29			
	Total	12	.2308	.03942	.01138	.2058	.2559	.16	.29			
	1	3	1.4067	.03512	.02028	1.3194	1.4939	1.37	1.44			
Lactic acid	7	3	1.3633	.00577	.00333	1.3490	1.3777	1.36	1.37			
concentration	14	3	1.3500	.02000	.01155	1.3003	1.3997	1.33	1.37			
(% w /v)	21	3	1.3233	.03512	.02028	1.2361	1.4106	1.29	1.36			
	Total	12	1.3608	.03895	.01125	1.3361	1.3856	1.29	1.44			
	1	3	2.6133	.02517	.01453	2.5508	2.6758	2.59	2.64			
Acetic acid	7	3	2.5200	.05000	.02887	2.3958	2.6442	2.47	2.57			
concentration	14	3	2.4800	.04000	.02309	2.3806	2.5794	2.44	2.52			
(% w /v)	21	3	2.1167	.00577	.00333	2.1023	2.1310	2.11	2.12			
	Total	12	2.4325	.19923	.05751	2.3059	2.5591	2.11	2.64			

Test of Homogeneity of Variances

	Levene Statistic	df1	df2	Sig.
Sucrose concentration (% w /v)	2.034	3	8	.188
Fructose concentration (% w /v)	.088	3	8	.964
Lactic acid concentration (% w /v)	1.388	3	8	.315
Acetic acid concentration (% w /v)	1.224	3	8	.363

Multiple Comparisons

Depende	ent		Mean	Std.	Sig	95% Confid	ence Interval
Storage o	days		Difference (I-J)	Error	org.	Lower Bound	Upper Bound
		7	.24667*	.02333	.000	.1929	.3005
	1	14	.62667*	.02333	.000	.5729	.6805
		21	1.36333 [*]	.02333	.000	1.3095	1.4171
		1	24667*	.02333	.000	3005	1929
Cuerese	7	14	.38000*	.02333	.000	.3262	.4338
Sucrose		21	1.11667^{*}	.02333	.000	1.0629	1.1705
(% w (v)		1	62667*	.02333	.000	6805	5729
(% \/ / /)	14	7	38000*	.02333	.000	4338	3262
		21	.73667*	.02333	.000	.6829	.7905
		1	-1.36333*	.02333	.000	-1.4171	-1.3095
	21	7	-1.11667*	.02333	.000	-1.1705	-1.0629
		14	73667*	.02333	.000	7905	6829
		7	04333	.02173	.081	0934	.0068
	1	14	06333 [*]	.02173	.019	1134	0132
		21	08333 [*]	.02173	.005	1334	0332
		1	.04333	.02173	.081	0068	.0934
Fruetoso	7	14	02000	.02173	.384	0701	.0301
Fructose		21	04000	.02173	.103	0901	.0101
(% w A)		1	.06333*	.02173	.019	.0132	.1134
(90 VV / V)	14	7	.02000	.02173	.384	0301	.0701
		21	02000	.02173	.384	0701	.0301
		1	.08333*	.02173	.005	.0332	.1334
	21	7	.04000	.02173	.103	0101	.0901
		14	.02000	.02173	.384	0301	.0701
		7	.04333	.02198	.084	0074	.0940
	1	14	.05667*	.02198	.033	.0060	.1074
		21	.08333*	.02198	.005	.0326	.1340
		1	04333	.02198	.084	0940	.0074
Lactic acid	7	14	.01333	.02198	.561	0374	.0640
concentration		21	.04000	.02198	.106	0107	.0907
(% w /v)		1	05667*	.02198	.033	1074	0060
(,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,	14	7	01333	.02198	.561	0640	.0374
		21	.02667	.02198	.260	0240	.0774
		1	08333*	.02198	.005	1340	0326
	21	7	04000	.02198	.106	0907	.0107
		14	02667	.02198	.260	0774	.0240
		7	.09333^	.02819	.011	.0283	.1583
	1	14	.13333*	.02819	.001	.0683	.1983
		21	.49667*	.02819	.000	.4317	.5617
		1	09333*	.02819	.011	1583	0283
	7	14	.04000	.02819	.194	0250	.1050
Acetic acid		21	40.3.33*	02819	000	3383	4683
concentration		1	12222*	02010	001	1092	0692
(% w /v)	14	⊥ ~	10000	02019	.001	1300	0003
	14	1	04000	.02818	.194	1050	.0250
		21	.36333	.02819	.000	.2983	.4283
		1	49667*	.02819	.000	5617	4317
	21	7	40333 [*]	.02819	.000	4683	3383
		14	36333*	.02819	.000	4283	2983

ANOVA									
		Sum of Squares	df	Mean Square	F	Sig.			
Sucrose concentration (% w /v)	Between Groups	3.185	3	1.062	1299.874	.000			
	Within Groups	.007	8	.001					
	Total	3.191	11						
Fructose	Between Groups	.011	3	.004	5.376	.025			
concentration (% w /v)	Within Groups	.006	8	.001					
	Total	.017	11						
Lactic acid concentration (% w /v)	Between Groups	.011	3	.004	5.008	.030			
	Within Groups	.006	8	.001					
	Total	.017	11						
Acetic acid	Between Groups	.427	3	.142	119.466	.000			
concentration (% w /v)	Within Groups	.010	8	.001					
	Total	.437	11						

F.3.6 Statistical analysis of protein content of fermented pea protein-coconut milk beverage during storage (4 $^{\circ}\rm{C}$) for 21 days

Descriptives

Protein concentration (%)

	N N	Std.		0.15	95% Confide for N	ence Interval <i>I</i> lean	Minimum	Maria	
		Mean	Deviation	SLU. EITOI	Lower Bound	Upper Bound	winimum	Maximum	
1	3	2.0000	.06000	.03464	1.8510	2.1490	1.94	2.06	
7	3	2.0200	.03464	.02000	1.9339	2.1061	2.00	2.06	
14	3	2.0400	.03464	.02000	1.9539	2.1261	2.00	2.06	
21	3	2.0833	.04041	.02333	1.9829	2.1837	2.06	2.13	
Total	12	2.0358	.04926	.01422	2.0045	2.0671	1.94	2.13	

Test of Homogeneity of Variances

Protein concentration (%)

Levene Statistic	df1	df2	Sig.
.288	3	8	.833

ANOVA

Protein concentration (%)

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.011	3	.004	1.996	.193
Within Groups	.015	8	.002		
Total	.027	11			

Multiple Comparisons

Dependent Variable: Protein concentration (%)

	LSD									
(I) Storage days		Mean	0	0.	95% Confidence Interval					
		Difference (I- J)	Std. Error	Sig.	Lower Bound	Upper Bound				
	7	02000	.03567	.590	1023	.0623				
1	14	04000	.03567	.295	1223	.0423				
	21	08333*	.03567	.048	1656	0011				
	1	.02000	.03567	.590	0623	.1023				
7	14	02000	.03567	.590	1023	.0623				
	21	06333	.03567	.114	1456	.0189				
	1	.04000	.03567	.295	0423	.1223				
14	7	.02000	.03567	.590	0623	.1023				
	21	04333	.03567	.259	1256	.0389				
	1	.08333*	.03567	.048	.0011	.1656				
21	7	.06333	.03567	.114	0189	.1456				
	14	.04333	.03567	.259	0389	.1256				

*. The mean difference is significant at the 0.05 level.

E.3.7 Statistical analysis of semi-trained sensory panel (n=15) sensory evaluation of fermented pea protein-coconut milk beverage during storage (4 $^{\circ}$ C) for 21 days

Test of Homogeneity of Variances								
	Levene Statistic	df1	df2	Sig.				
Appearance	1.477	3	56	.231				
Aroma	4.269	3	56	.009				
Sourness	3.538	3	56	.020				
Sweetness	2.051	3	56	.117				
Flavour	8.682	3	56	.000				
Mouth feel	4.304	3	56	.008				
After taste	2.222	3	56	.096				
Overall acceptability	4.000	3	56	.012				

Descriptives									
						95% Cor	nfidence		
		NI		Std.	Std.	Interval f	or Mean	N 41-1-1-1-1-1-1-1-1-1-1-1-1-1-1-1-1-1-1-	N 4
Store da	ays	IN	iviean	Deviation	Error	Lower	Upper	winimum	Iviaximum
						Bound	Bound		
	1	15	7.0000	1.25357	.32367	6.3058	7.6942	5.00	9.00
	7	15	6.8000	.67612	.17457	6.4256	7.1744	6.00	8.00
Appearance	14	15	6.6000	.82808	.21381	6.1414	7.0586	6.00	8.00
	21	15	6.0000	.92582	.23905	5.4873	6.5127	4.00	8.00
	Total	60	6.6000	.99490	.12844	6.3430	6.8570	4.00	9.00
	1	15	7.0000	1.30931	.33806	6.2749	7.7251	6.00	9.00
Aroma	7	15	6.8000	.67612	.17457	6.4256	7.1744	6.00	8.00
	14	15	7.0000	1.00000	.25820	6.4462	7.5538	5.00	8.00
	21	15	7.0000	.92582	.23905	6.4873	7.5127	5.00	8.00
	Total	60	6.9500	.98161	.12673	6.6964	7.2036	5.00	9.00
	1	15	5.0000	1.19523	.30861	4.3381	5.6619	4.00	7.00
	7	15	6.0667	.70373	.18170	5.6770	6.4564	5.00	7.00
Sourness	14	15	6.2000	1.20712	.31168	5.5315	6.8685	4.00	7.00
	21	15	6.4000	1.12122	.28950	5.7791	7.0209	5.00	8.00
	Total	60	5.9167	1.18310	.15274	5.6110	6.2223	4.00	8.00
	1	15	6.2000	1.01419	.26186	5.6384	6.7616	5.00	8.00
	7	15	6.4000	.91026	.23503	5.8959	6.9041	5.00	8.00
Sweetness	14	15	6.4000	.82808	.21381	5.9414	6.8586	5.00	7.00
	21	15	6.2000	.56061	.14475	5.8895	6.5105	5.00	7.00
	Total	60	6.3000	.82954	.10709	6.0857	6.5143	5.00	8.00
	1	15	6.0000	1.46385	.37796	5.1893	6.8107	4.00	8.00
	7	15	6.4000	.50709	.13093	6.1192	6.6808	6.00	7.00
Flavour	14	15	6.2000	.56061	.14475	5.8895	6.5105	5.00	7.00
	21	15	6.8000	.67612	.17457	6.4256	7.1744	5.00	8.00
	Total	60	6.3500	.91735	.11843	6.1130	6.5870	4.00	8.00
	1	15	6.2000	1.08233	.27946	5.6006	6.7994	4.00	7.00
	7	15	6.4000	1.50238	.38791	5.5680	7.2320	4.00	8.00
Mouth feel	14	15	6.4000	.50709	.13093	6.1192	6.6808	6.00	7.00
	21	15	7.2000	.94112	.24300	6.6788	7.7212	5.00	8.00
	Total	60	6.5500	1.11119	.14345	6.2629	6.8371	4.00	8.00
	1	15	6.8000	1.14642	.29601	6.1651	7.4349	6.00	9.00
	7	15	6.0000	1.00000	.25820	5.4462	6.5538	5.00	8.00
After taste	14	15	6.2000	1.20712	.31168	5.5315	6.8685	5.00	8.00
	21	15	7.0000	.84515	.21822	6.5320	7.4680	5.00	8.00
	Total	60	6.5000	1.11233	.14360	6.2127	6.7873	5.00	9.00
	1	15	6.8000	.94112	.24300	6.2788	7.3212	6.00	8.00
	7	15	6.6000	.73679	.19024	6.1920	7.0080	5.00	8.00
Overall	14	15	6.6000	.50709	.13093	6.3192	6.8808	6.00	7.00
acceptability	21	15	6.2000	.67612	.17457	5.8256	6.5744	5.00	7.00
	Total	60	6.5500	.74618	.09633	6.3572	6.7428	5.00	8.00

ANOVA									
		Sum of Squares	df	Mean Square	F	Sig.			
	Between Groups	8.400	3	2.800	3.136	.032			
Appearance	Within Groups	50.000	56	.893					
	Total	58.400	59						
	Between Groups	.450	3	.150	.149	.930			
Aroma	Within Groups	56.400	56	1.007					
	Total	56.850	59						
	Between Groups	17.650	3	5.883	5.074	.004			
Sourness	Within Groups	64.933	56	1.160					
	Total	82.583	59						
	Between Groups	.600	3	.200	.280	.840			
Sweetness	Within Groups	40.000	56	.714					
	Total	40.600	59						
	Between Groups	5.250	3	1.750	2.207	.097			
Flavour	Within Groups	44.400	56	.793					
	Total	49.650	59						
	Between Groups	8.850	3	2.950	2.581	.062			
Mouth feel	Within Groups	64.000	56	1.143					
	Total	72.850	59						
	Between Groups	10.200	3	3.400	3.032	.037			
After taste	Within Groups	62.800	56	1.121					
	Total	73.000	59						
Quorall	Between Groups	2.850	3	.950	1.773	.163			
acceptability	Within Groups	30.000	56	.536					
	Total	32.850	59						
Multiple Comparisons

					OEK Confidence		
Dependent Variable: Store days			Mean			95% Contidence	
			Difference	Std Error	Sia	Interval	
			(1-1)		o.g.	Lower	Upper
			(1.5)			Bound	Bound
		7	.20000	.34503	.564	4912	.8912
	1	14	40000	34503	251	- 2912	1 0912
	1	21	1.00000*	34503	005	3088	1 6012
		1	20000	24502	.003	.0000	4012
	7 14 21 1	14	20000	.34503	.504	8912	.4912
		14	.20000	.34503	.564	4912	.8912
Appearance		21	.80000	.34503	.024	.1088	1.4912
rippourantee		1	40000	.34503	.251	-1.0912	.2912
		7	20000	.34503	.564	8912	.4912
		21	.60000	.34503	.088	0912	1.2912
		1	-1.00000*	.34503	.005	-1.6912	3088
		7	80000*	.34503	.024	-1.4912	1088
		14	- 60000	34503	088	-1 2912	0912
		7	20000	36645	587	53/1	03/1
		1	.20000	26645	1,000	3341	7041
		14	0.00000	.30043	1.000	7341	.7341
		21	0.00000	.36645	1.000	/341	.7341
		1	20000	.36645	.587	9341	.5341
	7	14	20000	.36645	.587	9341	.5341
Aroma		21	20000	.36645	.587	9341	.5341
Aroma		1	0.00000	.36645	1.000	7341	.7341
	14	7	.20000	.36645	.587	5341	.9341
		21	0 00000	36645	1 000	- 7341	7341
	21	1	0,00000	36645	1,000	- 7341	7341
		7	20000	26645	E97	7041 5241	0241
		1	.20000	.30043	.567	5541	.9341
	1	14	0.00000	.30045	1.000	/341	.7341
		1	-1.06667	.39320	.009	-1.8543	2790
		14	-1.20000*	.39320	.003	-1.9877	4123
		21	-1.40000 [*]	.39320	.001	-2.1877	6123
	7	1	1.06667*	.39320	.009	.2790	1.8543
		14	13333	.39320	.736	9210	.6543
		21	33333	.39320	.400	-1.1210	.4543
Sourness	14	1	1 20000*	39320	003	4123	1 9877
		7	13333	39320	736	- 6543	9210
		21	20000	.00020	.130	0343	.5210
		1	20000	.39320	.013	9077	.3077
	21	1	1.40000	.39320	100.	.0123	2.1877
		1	.333333	.39320	.400	4543	1.1210
		14	.20000	.39320	.613	5877	.9877
Sweetness	1 7	7	20000	.30861	.520	8182	.4182
		14	20000	.30861	.520	8182	.4182
		21	0.00000	.30861	1.000	6182	.6182
		1	.20000	.30861	.520	4182	.8182
		14	0.00000	.30861	1.000	6182	.6182
		21	20000	30861	520	.0102	8182
		1	20000	20961	.520	4102	.0102
	14	1	.20000	.30001	.520	4102	.0102
		1	0.00000	.30861	1.000	0182	.0182
		21	.20000	.30861	.520	4182	.8182
	21	1	0.00000	.30861	1.000	6182	.6182
		7	20000	.30861	.520	8182	.4182
		14	20000	.30861	.520	8182	.4182
Flavour	1	7	40000	.32514	.224	-1.0513	.2513
		14	- 20000	32514	541	- 8513	4513
		<u>⊥</u> -⊤)21	_ 80000*	22514	017	_1 /512	_ 1/107
		۲ ۲	00000	.32314	.011	-1.4010	140/ 1.0E10
	7 14	1	.40000	.32514	.224	2513	1.0513
		14	.20000	.32514	.541	4513	.8513
		21	40000	.32514	.224	-1.0513	.2513
		1	.20000	.32514	.541	4513	.8513
		7	20000	.32514	.541	8513	.4513
		21	60000	.32514	.070	-1.2513	.0513

		1	.80000*	.32514	.017	.1487	1.4513
	21	7	.40000	.32514	.224	2513	1.0513
		14	.60000	.32514	.070	0513	1.2513
		7	20000	.39036	.610	9820	.5820
	1	14	20000	.39036	.610	9820	.5820
		21	-1.00000*	.39036	.013	-1.7820	2180
		1	.20000	.39036	.610	5820	.9820
Mouth fool	7	14	0.00000	.39036	1.000	7820	.7820
		21	80000*	.39036	.045	-1.5820	0180
Modeli icci		1	.20000	.39036	.610	5820	.9820
	14	7	0.00000	.39036	1.000	7820	.7820
		21	80000*	.39036	.045	-1.5820	0180
		1	1.00000*	.39036	.013	.2180	1.7820
	21	7	.80000*	.39036	.045	.0180	1.5820
		14	.80000*	.39036	.045	.0180	1.5820
		7	.80000*	.38668	.043	.0254	1.5746
	1	14	.60000	.38668	.126	1746	1.3746
		21	20000	.38668	.607	9746	.5746
		1	80000*	.38668	.043	-1.5746	0254
After taste	7	14	20000	.38668	.607	9746	.5746
		21	-1.00000*	.38668	.012	-1.7746	2254
		1	60000	.38668	.126	-1.3746	.1746
	14	7	.20000	.38668	.607	5746	.9746
		21	80000*	.38668	.043	-1.5746	0254
		1	.20000	.38668	.607	5746	.9746
	21	7	1.00000*	.38668	.012	.2254	1.7746
		14	.80000*	.38668	.043	.0254	1.5746
Overall		7	.20000	.26726	.457	3354	.7354
	1	14	.20000	.26726	.457	3354	.7354
		21	.60000*	.26726	.029	.0646	1.1354
		1	20000	.26726	.457	7354	.3354
	7	14	0.00000	.26726	1.000	5354	.5354
		21	.40000	.26726	.140	1354	.9354
acceptability		1	20000	.26726	.457	7354	.3354
	14	7	0.00000	.26726	1.000	5354	.5354
		21	.40000	.26726	.140	1354	.9354
		1	60000*	.26726	.029	-1.1354	0646
	21	7	40000	.26726	.140	9354	.1354
		14	40000	.26726	.140	9354	.1354

*. The mean difference is significant at the 0.05 level.